

THE MECHANISM OF THE ACTION OF INDOLE-3-ACETIC ACID ON THE WATER ABSORPTION BY AVENA COLEOPTILE SECTIONS

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GENERAL INTRODUCTION

The mechanism of the growth by cell extension has been a debatable point for many years, in fact since the early days of plantphysiology. The final visible outcome of cell extension is a permanent increase in cell volume, accompanied by a permanent increase in wall area. This seems to be a rather simple phenomenon. Further investigation, however, revealed the difficulty of establishing the exact sequence of events leading to these final results. The initiation of elongation was attributed to a host of different factors, for instance increase in wall elasticity (SACHS), active intussusception of wall substances (PFEFFER) etc. An extensive survey of this older literature is given by HEYN (1940).

After the discovery of the growth hormone by WENT (1928) and its subsequent isolation by KÖGL and HAAGEN SMIT (1931) the situation became even more complicated. The problem of the mechanism of the action of growth substances demanded a solution. The fact that in most experiments cell extension takes place almost exclusively by the absorption of water, was a lead for attempts in this field of research. Water enters a cell only as a response to a positive suction force. Therefore a positive suction force must be maintained during all stages of elongation. Growth substances may stimulate elongation by disturbing the dynamic equilibrium which exists in the cell. This effect can be attained along two different ways: an increase in the direct forces attracting water into the cell, for instance an increase in the osmotic value of the cell sap or an increase in the imbibitional forces of the cell colloids, or a decrease in the countering forces, for instance a lessening of the wall pressure, which is an inwardly directed hydrostatic pressure originating as a reaction of the elastically stretched cell wall.

The greater part of research has been centered about the latter possibility, which means a change of the properties of the cell wall. Important work has been done by HEYN (1931-1934, 1940). He found that the growth rate is connected with the plasticity of the cell wall. Growth was supposed to be mainly dependent on this property, which was regulated by growth substances. SÖDING (1931-1934, 1952) did not find such clear-cut correlations. He also maintained the im-

portance of the plasticity, but expressed as his opinion that the role of plasticity had been exaggerated.

The majority of the authors reject a change in elasticity as an initiator of growth. Some, however, are of the opinion that it may be important in the first phase of elongation: BURSTRÖM (1942, 1951).

Concerning the first mentioned possibility it can be said that only a few determinations of the suction force are available. The determinations of the osmotic value show that it is not increased through growth substances. A distinction must be made between the values found for intact seedlings and those determined on isolated tissues. In the latter the decrease in the osmotic value is more evident than in the former.

In later years the theories employing the action of growth substances on the cell wall appeared to give too simple a conception of the real facts. It was impossible to explain the optimum curve and the various actions of growth substances (correlative inhibition, root formation, cambium activation) with an effect on the cell wall alone. Moreover, FREY-WYSSLING (1945) and WIRTH (1946) found that a synthesis of protoplasm components and wall substances accompanies the elongation. Therefore protoplasmic activity joins in, and at least part of the action of growth substances should be localized in the protoplasm, which is supported by investigations on the influence of indole-acetic acid on the metabolic activity of living cells. A stimulating effect on respiration was demonstrated by KELLY (1947), ANKER (1951, 1953) and other investigators.

One important property of the living cell, the permeability, has not yet been touched upon. KONINGSBERGER (1942, 1947) and VELDSTRA (1944) introduced the hypothesis that growth substances act on the ectoplast and change the permeability to water. This is further supported by the relation between structure and action of growth substances. (VELDSTRA, 1944). Although VELDSTRA and BOOY (1949) and BOOY and VELDSTRA (1949) found that the theory is not quite satisfactory, the idea has not yet been dismissed that growth substances influence, as their primary effect, the protoplasmic properties through a physico-chemical action on its colloidal constituents. Recently ANKER (1953) suggested that growth substances affect inner lipophilic protoplasmic films.

In the present investigation the problem of the mechanism of the action of growth substances is approached by considering the existing possibilities for a change in the rate of water intake. It seemed worth while to carry out determinations of these factors on the same material, as which were chosen for *Avena* coleoptile sections. After establishing the experimental conditions the permeability to water was investigated. No increase in the permeability to water, which might facilitate the water absorption, was found.

Next an investigation was made into the relation between the presence of growth substances and the magnitude of the suction force, in combination with determinations of the osmotic value. These experiments showed that the osmotic value decreases in proportion to

the increase in length of the sections. Additionally, however, it appeared in these experiments that the wall properties must be changed after treatment with indole-3-acetic acid. A detailed study of this effect was made and the effect of substances, which influence metabolism, on the action of indole-acetic acid was examined. The results show, that a link exists between the metabolic activity and the change in wall properties. This means that the wall is changed by growth substances through the intermediary of the protoplasm. It is held that the synthesis of new wall materials is affected by growth substances. In view of other data available in the relevant literature it seems most likely that the action of growth substances is primarily a physico-chemical one. This is supported by the permeability experiments reported in this paper. The existence of an active absorption of water, as suggested by some authors, e.g. THIMANN (1951), is rejected because the available evidence is considered insufficient. Of course water uptake caused through an active growth of the cell wall is "active", as it takes energy, but it differs from the generally accepted concept of "active water absorption".

MATERIAL and GENERAL METHODS

The experiments were carried out with sections excised from the coleoptiles of four-day-old etiolated seedlings of *Avena sativa*, variety "Victory" (Sveriges Utsädeförening, Svalöf, Sweden). The unhusked seeds were soaked in tap water for one hour and grown in moist sawdust in complete darkness at 23° C and 96 % relative humidity. All manipulations were carried out in the same darkroom in orange light (Schott OG2 filter), which is phototropically inactive.

Generally the used coleoptiles had a length of about 2 cm. They were placed in a coleoptile microtome after VAN DER WEY (1932) and 4-mm tips were cut and rejected. The next 2½-mm sections (4–6½ mm from the tip) were employed in the experiments. The primary leaf was removed before or after cutting the sections. In the latter case the primary leaf was pushed out of the sections with a thin glass rod, while in the former case the procedure described by VAN DER WEY (1932) was used: part of the base of the coleoptile was removed with a pair of decapitation scissors, after which the primary leaf could be pulled out. Unless otherwise stated these 2½-mm sections without primary leaf were used.

The permeability determinations were carried out with the electromagnetic diver method described by BUFFEL (1952). A detailed description is given in chapter 2. The heavy water (D₂O) used in these experiments was supplied by the Norsk Hydro-elektrisk Kvaelfstofaktieselskab, Oslo, Norway.

In the greater part of the experiments the section test after BONNER (1933) was employed, as modified by VAN SANTEN (1940) and RIETSEMA (1949, 1950). Thus the sections were placed on a slide by means of a narrow vaseline strip and the solutions were aerated during the test. This technique was adapted, when necessary, to the

needs of this investigation. Generally the test was performed without the use of buffer mixtures to keep circumstances as simple as possible. More detailed methodical data can be found in the different chapters.

Two different indole-3-acetic acid (I.A.A.) preparations were available, one from Schering-Kahlbaum (Berlin) and the other one from the Amsterdamse Kinine Fabriek (Amsterdam). The indole-acetic acid was solved by means of gentle heating.

The water used in the experiments was distilled over glass.

CHAPTER I

WATER ABSORPTION

1. LITERATURE

The work on the influence of I.A.A. on the intake of water by tissues has been mainly restricted to storage tissues. Only a few investigators included actively growing tissues in their experiments. Conclusions valid for these storage tissues do not necessarily hold for growing tissues, although it seems that they do. This should be well borne in mind.

This phenomenon has been discovered by REINDERS (1938, 1942). She found that water absorption by potato tuber discs is an aerobic process, promoted by I.A.A. which also causes an increased loss in dry weight. The influence of I.A.A. was found in distilled water. REINDERS proposed as probable causes a stimulation of the respiration, which might bring about an increased osmotic value, or a possible greater extensibility of the wall.

Her results were confirmed by other authors and the experiments were continued with a view to elucidating the mechanism of action of I.A.A. COMMONER and MAZIA (1942) thought about an increase in the osmotic value of the cell sap. This could be brought about through anatonosis or endosmosis. They stressed the latter possibility and tried to find a correlation between salt uptake and water uptake. Both processes were promoted by I.A.A. to the same degree. The authors obtained these results both with potato tuber discs and *Avena* coleoptile sections. A further analysis by COMMONER, FOGEL and MULLER (1943) in combination with the results of other investigators led to the hypothesis, that I.A.A. stimulates the respiration. This would produce an increased salt uptake which in its turn is compensated by an increased water uptake. The final result will be an increase in cell volume. COMMONER and MAZIA (1944) gave further data supporting this supposition for *Avena* coleoptiles and for potato tuber discs. They found an influence of C_4 -acids in the presence of I.A.A. on salt and water uptake.

This conception, however, seems to be too simple. The exact time sequence for salt and water uptake was not determined and compared. There is no proof that the hypothesis is quantitatively adequate and it does not explain the promoting effect of I.A.A. in distilled water as found by REINDERS (1942) and VAN OVERBEEK (1944). VAN

OVERBEEK criticizes the work of COMMONER et al. because they performed experiments with sucrose for 4 days without maintaining aseptic conditions. VAN OVERBEEK determined the osmotic value, which appeared to decrease slightly during the experiment, both with growth substance and without. The freezing point depression is lower in the discs treated with growth substance than in the controls, but the differences are rather small. These determinations were naturally carried out with press sap of tissues and are, therefore, not quite satisfactory. This author eliminates the role of starch hydrolysis with the argument that the osmotic value does not increase. This is premature for he did not carry out determinations of the amount of osmotic active material in the cell. Even then there remains the possibility that the greater part of the lower carbohydrates originating from starch hydrolysis has been consumed in respiration. VAN OVERBEEK considers a decrease in wall pressure or an increase in "the anomalous components of the suction force" as most important, the latter being the most probable cause.

LEVITT (1948) gives a thermodynamical consideration of the phenomenon and a series of experiments.

From the results of his experiments he deduces that an active absorption can be eliminated. LEVITT found water uptake at low temperatures (0° – 2° C) and with KCN, and not a loss of water as he had expected in the case of active absorption alone. This author supposes that I.A.A. increases the plasticity of the cell wall. He confirms VAN OVERBEEK's results in also finding lower freezing point depressions in discs treated with I.A.A.

All experiments show that the enhanced water uptake is connected with respiration. Without oxygen there is no water uptake. This is clearly demonstrated by KELLY (1947), who determined water uptake and respiration in the oat coleoptile. Azide, cyanide and iodoacetate inhibited both processes to the same degree. This is at variance with the results of LEVITT (1948). The stimulating action of I.A.A. was prevented by azide and iodoacetate.

HACKETT and THIMANN (1950, 1952) also studied the effect of various inhibitors: azide, arsenite, dinitrophenol and fluoroacetate. All these substances inhibit the water uptake. I.A.A. and naphthalene acetic acid promote the process, the latter being more effective. Reduction of the oxygen pressure also reduces the uptake of water. The authors conclude that the water uptake of potato tissue both in the presence and in the absence of externally added growth substance is linked to the general aerobic metabolism and corresponds closely to elongation in etiolated seedlings.

THIMANN (1951) suggested that the enhanced water uptake might be caused through a greater plasticity of the cell wall, brought about by an oxidative process, or by an active pumping of water into the cell. He prefers the latter possibility, considering that, after growth inhibition through immersion of the tissues in a solution with a fairly high osmotic value containing a growth substance, the growth rate does not increase suddenly when this solution is replaced by distilled

water also containing growth substance. This conclusion is not justified if the wall meanwhile has been changed because new material has been formed and interwoven. In that case secondary layers may have been laid down, solidifying the new parts of the primary wall. The same process will take place in cells inhibited by an external suction force and when this force is removed no sudden increase in growth rate can be expected. THIMANN's conclusion would be correct if the change in wall properties is brought about through a lasting loosening of the micellar structure.

HACKETT and THIMANN (1953) studied the relation between respiration and water absorption. They used dinitrophenol as an inhibitor and they concluded that water uptake is dependent on a sufficient supply of high-energy phosphate bonds. This conclusion is supported by BONNER and BANDURSKI (1952). HACKETT and THIMANN find a relation between water uptake and respiration, but not between the rate of water absorption and the rate of respiration. The relation between both processes is not yet clear.

HACKETT (1952) determined the osmotic changes during water uptake of potato tissue cryoscopically. The effect of naphthalene acetic acid on the fresh weight and on the shift of the freezing point depression appeared to match.

This means that the osmotic value of the cell sap decreases in direct proportion to the increase in fresh weight. The results of VAN OVERBEEK (1944) and LEVITT (1948) are thus confirmed.

A close relation between water intake and oxybiosis was also established by BRAUNER, BRAUNER and HASMAN (1940) and by BRAUNER and BRAUNER (1943).

HSIANG (1951) studied the water uptake by discs cut from the floral parts of orchids. She found an influence of growth substances and a dependency of water absorption on an aerobic process.

AUDUS (1952) criticizes the results of many other investigators. He stresses that it is necessary for an exact interpretation of experiments about the action of inhibitors to compare growth rates and not total growth determinations. This is the more important when the influence of inhibitors on two different processes, for instance respiration and growth, is compared in order to establish a relation between these two processes. In this respect the results of many authors must be reconsidered.

Frequently the influence of different substances on water uptake, or growth, and their effect on respiration are not determined after the same time of treatment. A comparison of the values found will give an erroneous impression of the relation between both processes. AUDUS shows this for the action of arsenite on growth (water uptake) and respiration in pea stem sections.

The evidence surrounding the effect of growth substances on the water intake of tissues is thus very conflicting. The connection of the absorption of water with the respiration is evident, but the exact relation between the two processes is uncertain. There is as yet insufficient direct proof that oxygen and respiration affect an active

water absorption. It is possible that the metabolic activity changes the wall properties and that the greater water uptake can be accounted for osmotically. Some authors point out that the permeability to water can be affected: KONINGSBERGER (1942, 1947), VELDSTRA (1944), VON GUTTENBERG and KRÖPELIN (1947), POHL (1948) and BRAUNER and HASMAN (1949). The greater water permeability however can explain only a difference in rate of uptake and is not able to shift the final equilibrium. Moreover, the permeability to water is already relatively great. Therefore a change in permeability to water cannot be considered as the primary cause of the increase in water absorption.

2. EXPERIMENTS

Water uptake can be measured as the increase in fresh weight of a number of isolated coleoptile sections. A series of experiments was carried out with $2\frac{1}{2}$ -mm sections without primary leaf. 30 sections were cut, placed on thin glass rods in 100 ml distilled water in a Petri dish and aerated. The rods with sections were kept under the surface by sticking them to the bottom of the dish with vaseline. After 6 hours the sections were lifted out from the dishes, blotted and weighed and then put in the experimental solutions. After 24 hours the sections were weighed again and the increase the fresh weight was determined. The results shown in table 1 are the average values from 6 experiments.

TABLE 1.
The effect of indole acetic acid on water absorption.

	Total weight mg		Weight per section mg		Increase mg per section	Increase %
	Initial	Final	Initial	Final		
Contr.	62,1	66,6	2,07	2,22	0,15	7,2
1 mg/l	61,5	77,9	2,05	2,60	0,55	26,8
10 mg/l.	63,4	87,8	2,11	2,93	0,82	38,9

The water uptake is enhanced by I.A.A., the highest concentration having the greatest effect. After these preliminary experiments it seemed desirable to determine the I.A.A. concentration that has the maximum effect. Therefore the technique was changed in a manner which allowed more I.A.A. concentrations to be tested in the same experiment.

$7\frac{1}{2}$ -mm sections (4– $11\frac{1}{2}$ mm from the tip) were cut, the primary leaf removed and 30–50 sections floated on the surface of 100 ml distilled water in a Petri dish. After 6 hours the sections were blotted, weighed and placed in the experimental solutions (250 ml). In order to keep the sections under the surface of the solution, they were enclosed in a glass vial (height $3\frac{1}{2}$ cm, diameter $4\frac{1}{2}$ cm), the open ends of the tubes being closed with cheese cloth. The solutions were aerated through a Jena G1 glass filter. After 24 hours the increase in fresh weight was determined. Special care was taken to make sure that the solution was removed from the central hole of the sections

TABLE 2
The increase of the fresh weight of sections under influence of I.A.A. in % per section.

I	II	III	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻²	10 ⁻¹	0,5	1	5	10	20	30	40	50	60	100
1*	6	30	37,7										75,4						
2†	23	32	6,0						76,1		78,8		7,2						
3†	6	30							4,4		10,2								
4	6	50							45,2	49,5	60,6	62,1	57,9			57,8	45,2		29,3
5†	6	50									48,4	46,1	50,7			52,1	45,1		11,1
											44,5								
6	6	40	18,6						21,4		37,8		43,7			42,7		45,6	
7	6	40											20,4	21,3		18,7	19,0		
8	6	40	18,8										54,5	55,6		47,6		29,8	
9	6	40	10,4					19,8	24,0		41,6		39,2						
10	6	40	9,4		9,5	10,4	9,6												
11	6	50	9,5	10,2	9,4	7,8	10,9												
12§	6	50	10,8		8,0	11,8	10,4	14,8											

I. Number of the experiment.

II. Time in hours during which the sections remained in distilled water before the experiment.

III. Number of sections per series.

† Sections with primary leaf. § 5-mm sections.

* Plants as are grown for the standard Avena test (page 404). The upper row of the table, above the heavy line, shows the concentration of I.A.A. in mg/l.

before weighing. The results of these experiments are collected in table 2.

Table 2 shows that the maximum effect was generally found between 1 and 40 mg/l, with a preference for the higher concentrations. This confirms the results of COMMONER and MAZIA (1942) and agrees more or less to the data of KELLY (1947). The lowest concentration of I.A.A. giving a stimulation of the water uptake is found to be 0.01 mg/l. Further dilutions had no significant effect.

In the greater part of the experiments high concentrations of I.A.A. appeared to be injurious. Fig. 1 shows this very clearly for concentrations higher than 10 mg/l. The sections become twisted and partly flaccid. The sensitivity to I.A.A. differed from one batch of plants

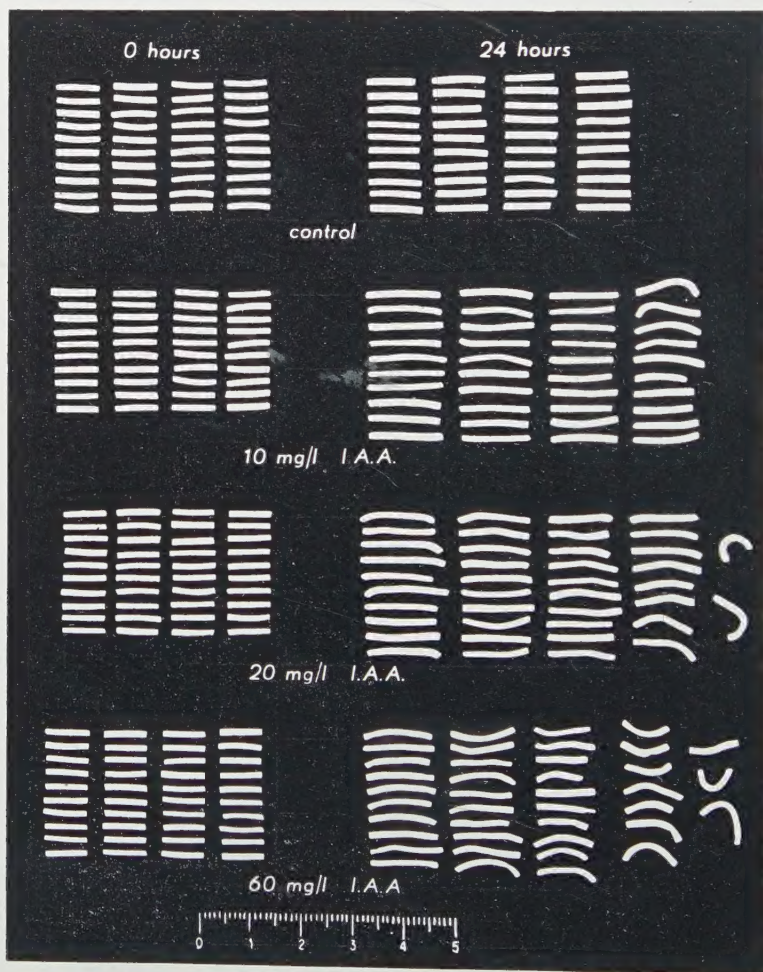


Fig. 1. The injurious effect of high concentrations of I.A.A.

to another. In one experiment (exp. 6 for example) the maximum effect was found at 60 mg/l, while in all the other experiments this concentration was definitely injurious. Therefore in the following experiments 1 and 10 mg/l will be used almost exclusively.

In some experiments the effect of coumarin was tested, sometimes combined with I.A.A. (Table 3).

TABLE 3
The effect of I.A.A. and coumarin on the procentual increase of fresh weight

	Exp. 1	Exp. 2
Control	4,3	15,2
Coumarin 1000 mg/l.	1,3	6,6
Coumarin 500 mg/l.		6,6
Coumarin 100 mg/l.	4,1	9,7
I.A.A. 10 mg/l	25,0	
I.A.A. 10 mg/l plus coumarin 100 mg/l. .	11,0	

The representative experiments in table 3 show that coumarin had an inhibitory influence on the uptake of water and partly suppressed the stimulating action of I.A.A. The same was found when elongation was measured as an indicator for the effect of coumarin (table 4).

TABLE 4
The effect of I.A.A. and coumarin on elongation

I.A.A.	Coumarin	Elongation
—	—	13,3 %
10 mg/l	—	55,8 %
—	100 mg/l	10,4 %
10 mg/l	100 mg/l	18,0 %
—	500 mg/l	12,3 %
10 mg/l	500 mg/l	15,0 %

The concentrations showing inhibition are of the same magnitude as those used by VON GUTTENBERG and BEYTHIEN (1951) in their permeability studies with epidermal cells of *Rhoeo discolor*.

3. DISCUSSION

The experiments described above confirm the facts found by other investigators. I.A.A. strongly promotes the uptake of water, while coumarin has an inhibiting effect. The most favourable conditions for further experiments can be derived from these results. So the most suitable concentration of I.A.A. will be 1 or 10 mg/l. It is also shown that a considerable absorption of water takes place in distilled water. Therefore the experiments can be performed in a very simple medium, no sugars or salts being required. This simplifies the interpretation of the results.

I.A.A. may bring about the increase in water uptake through an increase in the osmotic value or a decrease in the wall pressure as purely osmotic possibilities, or through the stimulation of an active

water absorbing mechanism. A change of the permeability may affect the rate of uptake. The permeability to water will be examined in the next chapter.

CHAPTER II

THE PERMEABILITY TO WATER

A. INTRODUCTION

From the experiments described in the preceding chapter the conclusion was drawn that I.A.A. enhances the absorption of water by isolated coleoptile sections. In several publications it has of late been suggested that growth substances may affect the permeability to water. Without being the primary cause (page 394) an increase in the permeability may facilitate the water uptake. This can be so only when the permeability functions as a "bottleneck" for the water absorption.

Growth and water uptake of isolated tissues are influenced, for example, by the pH of the medium and by the presence of growth substances. The following survey of the relevant literature discloses that no sufficient data about the effect of pH and growth substances on the permeability of growing tissues are yet available. We will examine, therefore, whether these two factors change the permeability to water. Even if no effect is found, this does not exclude the possibility that growth substances affect lipophilic membranes within the cells, but only the ectoplasm is at the moment accessible to experimental analysis.

B. LITERATURE

1. *pH and the permeability to water*

The influence of pH has been investigated by BRAUNER (1943). BRAUNER found a maximum permeability at an acid pH. He concluded that the optimum pH coincides with the I.E.P. of the protoplasm proteins.

POHL (1948) carried out investigations into the effect of pH on the permeability of *Avena* coleoptile sections. Different pH values were obtained with acetate buffer mixtures after MICHAELIS. These mixtures were diluted 1 : 10. POHL found maximum permeability at pH 4.3. However, the use of acetate buffers must be criticized because it is a well-known fact that acetate is toxic for plant tissues: COGGESHALL (1931), SAKAMURA and KANAMORI (1935), VAN SANTEN (1940), LUNDEGÅRDH (1942). VAN SANTEN proved that even a 1/3000 N acetate buffer damaged the roots of *Helianthus annuus*. The injurious effect increased with increasing acidity. POHL's use of citrate-phosphate mixtures in other experiments does not do away with this objection, for VAN SANTEN (1940) reported that citrate is also toxic.

SEEMANN (1949) studied the effect of pH on the permeability to

water of different adult tissues. He found a single peaked curve, with an optimum at pH 6 or 7. Generally, a plateau region of maximum permeability was found and not a distinct optimum, while the permeability decreased in low and high pH. He could not decide between an optimum corresponding to the I.E.P. of the protoplasmic proteins or an optimum coinciding with neutrality.

VON GUTTENBERG and MEINL (1952) carried out experiments with the epidermal cells of *Rhodo discolor*. They determined the pH of different I.A.A. solutions and compared the effect of these solutions with the effect of different pH's obtained by adding HCl to distilled water. The greatest permeability was found at pH 5.4. pH 3.7 decreased the permeability but did not kill the cells as did the I.A.A. solutions with a comparable pH (1 gr/l). The experiments were repeated with citric acid over a pH range from 1.3–5.8. In this solution the maximum permeability was found at pH 5.6–5.8. This result does not signify much, because there are no data available about the effect of neutral and alkaline pH. VON GUTTENBERG and MEINL also studied the effect of a di-potassiumcitrate buffer after SÖRENSEN. They found a maximum permeability at pH 5.4–5.6. It seems more in agreement with their data to state that not much difference exists between pH 4.4 and 6.0.

It appears from this survey of the relevant literature that only POHL (1948) carried out permeability determinations with growing tissues and his experiments are not quite convincing. Therefore it seemed worth while to examine the effect of pH on the permeability of growing tissues once more.

2. Growth substances and the permeability to water

KONINGSBERGER (1942, 1947) and VELDSTRA (1944) suggested that the primary effect of growth substances might be found in the ectoplasm. They considered the ectoplasm as a complexcoacervate film in the sense of BUNGENBERG DE JONG (1947). Growth substances might act as sensitizers when they are absorbed in the ectoplasm. The result would be a change of permeability.

KONINGSBERGER and co-workers (1947) supported this hypothesis with evidence from studies about the effect of I.A.A. on the rate of deplasmolysis of isolated protoplasts, obtained from the mesophyll cells of the bulb scales of *Allium cepa* (the onion). 0.1 and 1 mg/l I.A.A. blocked up the permeability to water completely for a few minutes. When the permeability was restored it remained decreased for 1–2 hours. This indicated that I.A.A. affects the protoplasmic membrane.

VELDSTRA (1944) suggested on the ground of his experiments about the relation between structure and activity of growth substances, that growth substances may have a sensitizing effect on coacervates. BOOY and VELDSTRA (1949) and VELDSTRA and BOOY (1949) carried out experiments to test this hypothesis and therefore studied the effect of different growth regulators on coacervates. The type of reaction found met with the expectations, but the quantitative

relations between the opening effect on coacervates and the physiological activity in the pea test were the reverse of those expected. The same was found in the "beet test" employed by VELDSTRA. Therefore the authors were led to consider the possible effect of growth regulators on the ectoplasmic layer as not being the primary cause of the growth reaction. Their experiments show clearly, however, that growth substances are membrane-active.

VON GUTTENBERG and KRÖPELIN (1947) investigated the influence of I.A.A. on the nyctinastic movements of the leaves of *Phaseolus coccineus*. The authors suggested that I.A.A. increases the permeability to water in the swelling tissue of the motile pulvini. Experiments with *Rhoeo discolor* and *Allium cepa* confirmed that the permeability to water is increased under influence of I.A.A.

VON GUTTENBERG and BEYTHIEN (1951) carried out more extensive investigations about the effect of different growth substances, growth inhibitors and some ions on the permeability to water of epidermal cells of *Rhoeo discolor*. When I.A.A. is added during deplasmolysis, the permeability to water is decreased, which is in accordance with the findings of KONINGSBERGER et al. (1947). This inhibition is strongest with 0.1 mg/l I.A.A., but is more persistent at the higher concentrations of I.A.A. The authors stated that plasmolysis is also accelerated through I.A.A.

VON GUTTENBERG and MEINL (1952) tried to analyse the action of I.A.A. They excluded the possibility that the action of I.A.A. is an effect of pH. They suggest an effect of growth substances on the protein components of the protoplasm. But the authors base their conclusions on insufficient and unconvincing experimental data. The results of their swelling experiments do not justify the explanations given.

BRAUNER and HASMAN (1949) studied the water uptake of potato tissue. From a graphical analysis of gravimetric measurements of the suction force they arrived at the conclusion that after short times of exposure to I.A.A. the primary effect of I.A.A. consists in an enhancement of the water permeability, while after longer times of exposure the tensility of the wall has been increased. Against these experiments the objection may be put forward that the graphs used for the analysis have been drawn through three points only.

RUGE (1937) had already found that the permeability is increased after treatment with I.A.A. He found this effect when a considerable amount of elongation had occurred and consequently concluded that the change in permeability is a secondary effect.

The preceding investigations were carried out with adult tissues, which is a disadvantage when growth substances are studied. POHL (1948) made experiments with *Avena* coleoptile sections. He used the osmotic principle for his permeability determinations, dehydrating sections without actually plasmolysing them. POHL interprets different changes of length during a certain time of dehydration after an I.A.A. treatment as differences in permeability. This is not allowed without further consideration, because osmotic conditions or wall properties

may be changed by the action of I.A.A. Experiments to be described in Chapter III and IV demonstrate that this is indeed true, even after 1 hour of treatment with I.A.A.

For the relation between the concentration of I.A.A. and the resulting growth POHL found a double peaked curve during the first hour or so after the addition of I.A.A. POHL explained the first peak found at about 0.08 mg/l I.A.A. as a result of an increase in the permeability to water. Higher concentrations decreased the permeability to water, but then electro-osmosis would play a part.

Apart from the fact that a change of permeability cannot shift the final equilibrium, objections can be raised against the use of sections immediately after cutting. RIETSEMA (1950) found that a passive water uptake of an osmotic nature plays a prominent part during the first few hours after the immersion of freshly cut sections into a solution, because the sections are not fully turgescient. Conclusions based on measurements during this period must be regarded with extreme caution. In our own experiments (unpublished) we did not find a double peaked curve when using sections which had been immersed in distilled water for 5 hours before the addition of I.A.A.

BUFFEL (1949, 1952) studied the effect of I.A.A. on the permeability to heavy water of isolated *Avena* coleoptile sections. By employing heavy water BUFFEL was able to avoid the use of the osmotic methods (see page 402). He used "aged" sections, this means, sections which have been immersed in distilled water for about 18 hours before they are used in the experiment. The result of these experiments was that I.A.A. has an opening effect of about 20 % in concentrations of 0, 1, 1 and 10 mg/l. The increase in permeability is thus very slight and it seems unlikely that this small response can bring about the enormous rise in growth rate. Moreover, it will be desirable to know the response of sections which have not been aged and still show the full growth response.

C. METHODS

1. Introduction

The osmotic methods available for the investigation of the permeability to water were rather unsatisfactory. When plasmolysis and deplasmolysis occur injury to the cell is very likely (REINHARDT, 1899. ALBACH, 1930). Some authors avoid plasmolysis, but even then the hydration of the protoplasm and the area or thickness of the membranes will change, which may bring about an altered permeability (HUBER and HÖFLER, 1930. RESÜHR, 1935). Moreover, it is difficult to compare the permeability constant for water with those for other substances because the former is measured as a filtration constant, while the latter are diffusion constants.

The discovery of the heavy hydrogen isotope by UREY, BRICKWEDDE and MURPHY (1932) and its subsequent concentration in the form of heavy water by LEWIS and MACDONALD (1933) offered new possibilities, for D_2O allows a direct determination of the water concen-

tration and consequently of the permeability to water (WARTIOVAARA, 1944). D_2O was introduced in permeability studies by LUCKÉ and HARVEY (1935), followed for instance by KROGH and USSING (1937). WARTIOVAARA (1944) was the first who employed heavy water in permeability experiments with plants (*Tolypellopsis*).

The most serious objection to the use of D_2O is the possibility that the permeability (diffusion) constants for ordinary and for heavy water may be different. Early experiments seemed to support this possibility (BROOKS, 1935. PARPART, 1935.), but later investigations show that this is not correct. Both constants are the same within the experimental variation (WARTIOVAARA, 1944. LOVTROP and PIGOŃ, 1951).

BUFFEL (1949, 1952) developed three different methods for measuring the permeability to heavy water, which methods make use of the changes in reduced weight originating from the exchange of D_2O against H_2O . PIGOŃ and ZEUTHEN (1951) developed a method on the same principle, adapting the Cartesian diver balance after ZEUTHEN (1948) for the purpose.

2. *The electromagnetic diver method*

The method has been described extensively by BUFFEL (1952). Very light glass divers (fig. 2), weighing about 90 mg, their length being about 115 mm, bear a soft iron grain (9) enclosed in the lower end. Below the iron grain the diver (6) is drawn out to a very thin point, which rests on the smooth surface of a glass rod (8) in the narrow part of the diffusion vessel (5). This part of the diffusion vessel passes through the centre part of a solenoid (7). When current passes the coil an electromagnetic field is created, which magnetizes the iron grain of the diver. The iron grain is lifted up and the diver moves upward.

The force exercised upon the diver is, for a given solenoid, proportional to the strength of the current passing through the coil. The force which will be able to lift up the diver must be greater when the reduced weight of the diver is greater. Therefore, the current strength is a measure for the reduced weight.

This principle is worked out in the apparatus in the following way: The solenoid forms part of a circuit which consists of a rheostat and a slide wire resistance for regulating the current strength, an ammeter measuring the current in mA and a telegraph key which allows a rapid making and breaking of the circuit. The current is derived from a battery or from a number of dry cells connected in series.

A number of coleoptile sections without primary leaf and loaded with D_2O is slipped over the narrow upper part of the diver. The diver is put in the diver vessel, which is placed in a water bath at $23^\circ C$, its narrow part passing through the coil. The rubber stopper (1) is placed in position and the vessel is slowly filled through a rubber tube (2) from a separatory funnel with a solution not containing any heavy water. The D_2O in the sections is exchanged for ordinary water

and, as a result, the reduced weight of diver plus sections diminishes. By means of the rheostat and the slide wire resistance a current is obtained that creates a magnetic force which is just unable to lift the diver. The ammeter is read and the current strength recorded. The lower part of the diver is observed with a magnifying glass

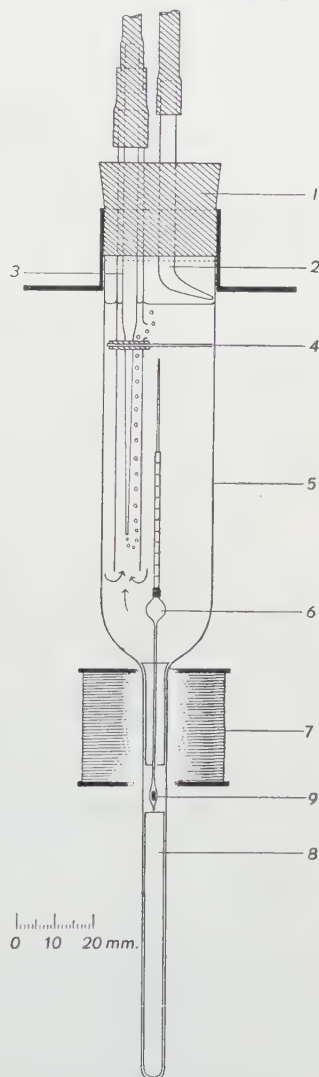


Fig. 2. Diver vessel with diver shown in detail.

1. rubber stopper.
2. filling tube.
3. aerating device.
4. mica plate.
5. diffusion vessel.
6. diver loaded with sections.
7. solenoid.
8. glass rod within the vessel.
9. iron grain.

during the measurements and the aeration is stopped. Measurements can easily be made at one-minute intervals. Care must be taken to measure the initial current strength as quickly as possible. The method is very sensitive and measures reduced weight differences of a few hundredths of mgs.

Each diver - vessel - solenoid combination must be calibrated for reduced weight as a function of current strength. This can be done by loading the divers with small spirals of nichrome resistance wire of known weight and measuring the lifting current. With the help of the calibration curves thus obtained reduced weights can be calculated from the recorded values of the current strength. Fig. 3

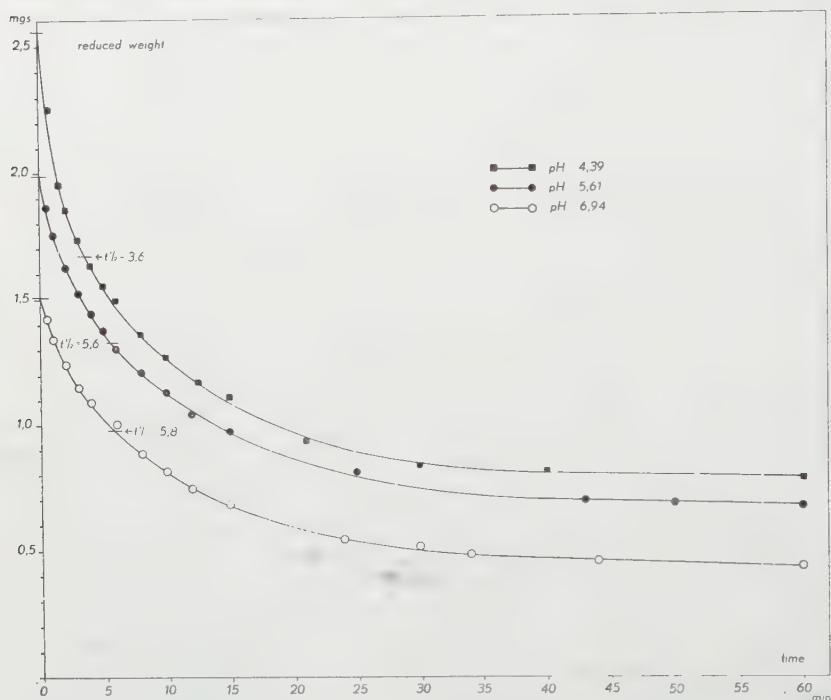


Fig. 3. Diffusion curves obtained with the electromagnetic diver method.

shows diffusion curves obtained in this way with the electromagnetic diver method. For a justified use of the calibration curves it is very important that the distance from the underside of the solenoid to the surface of the glass rod (8) remains constant.

The movement in the immersion fluid is very slight. Therefore a thorough aeration and mixing is necessary. This is accomplished by the device (3) shown in fig. 2. The small mica plate (4) attached to the aerating device serves the purpose of preventing excessive upward movements of the diver.

3. The preparation of the sections

For the permeability experiments plants were grown in waterculture as for the standard *Avena* test. This is briefly as follows:

Seeds were husked, soaked and laid out for 24 hours on moist filterpaper in a darkroom. Next, the germinated seeds were placed in glass holders, their roots in water. Two days later the plants were

ready for use. During the three days of cultivation the plants remained in the same darkroom at 23° C and 96 % relative humidity.

The procedure for cutting the sections has been described on page 390. The sections are infiltrated with water or a buffer mixture by centrifuging for 5 minutes at a rate giving the sections an acceleration of 500 times that of gravity. After infiltration the sections were kept in the darkroom for 5 hours in an amount of fluid corresponding to 2½ ml per section and aerated. The composition of this solution can be varied according to the purpose of the experiments.

After 5 hours the sections were blotted carefully and 12 sections were placed for one hour in a small flask containing ½ ml 50 % D₂O. During this hour the sections exchanged part of their ordinary water for heavy water. After 1 hour an equilibrium was established (BUFFEL, 1949) and the sections were lifted out from the flasks, blotted and placed on the divers, 12 sections per diver. The sequel of the experiment has been described in the preceding section.

The 50 % solution of heavy water was prepared through dilution from about 99,7 % D₂O. This dilution can be effected with distilled water, buffer mixtures or with solutions containing growth substances, according to the needs of the experiment.

Buffer mixtures were prepared from 1/100 mol.KH₂PO₄ and 1/100 mol.K₂HPO₄. For pH values below 4,5 1/100 mol.H₃PO₄ was added.

4. *The influence of heavy water on growth*

In the years after the discovery of the heavy water many experiments have been carried out about the effect of D₂O on the activities of the living cell. The results differ. BARNES (1934) and MEYER (1934) found that *Euglena gracilis* and *Aspergillus* grew better in solutions containing small amounts of D₂O. LEWIS (1934) found that seed germination is impossible in pure D₂O and 50 % inhibited in 50 % D₂O. PACSU (1934) found that the alcoholic fermentation of d-glucose is retarded in about 100 % D₂O, while CALDWELL, DOEBELING and MANIAN (1936) found a higher inactivation of pancreatic amylase in about 100 % heavy water. LEWIS (1934) concluded on the ground of the experiments known to him, that D₂O is not very toxic, but only slows down the activities of the cell more or less in direct proportion to the % D on the total H present.

BUFFEL (1952) observed at the outset a stimulating effect of 50 % D₂O on the growth of *Avena* coleoptile sections with 1 % sucrose and 1 mg/l I.A.A. After 12 hours in D₂O however the sections were flaccid.

WARTIOVAARA (1944) found that even concentrations of about 100 % D₂O apparently do not damage the cells of *Tolypellopsis stelligera*. In 50 % D₂O the cells remained alive for many days, while plasma rotation continued.

Many other investigations might be cited showing these phenomena. Part of these experiments has been carried out with inadequate techniques or the results are not supported by a sufficient number of observations. Many results, especially from the period immediately

after the isolation of heavy hydrogen, can be discarded because of insufficiently purified D₂O. In later years the quality of the D₂O preparations has been improved. Other investigators used freshly distilled heavy water. It is common knowledge that freshly distilled water contains much toxic mono- and dihydrol, which substances inhibit growth, while melted ice contains the much more favourable trihydrol (LLOYD and BARNES, 1932). The mechanism of the action of D₂O is not yet understood, but it is not necessarily a specific toxic action of the deuterium (FRERICKS, 1934). A slower uptake may also play a part.

We carried out some experiments in order to establish whether heavy water has an injurious effect on the sections in the permeability experiments. Therefore sections were placed in distilled water, 50 % and 100 % D₂O immediately after cutting. Ten sections were placed in small Petri dishes in 3 ml solution, no I.A.A. being added. The heavy water for this experiment had been distilled from alkaline permanganate some time before. Table 5 and fig. 4 show the results. D₂O appears to inhibit partly the autonomous growth. This is very evident in the highest concentration.

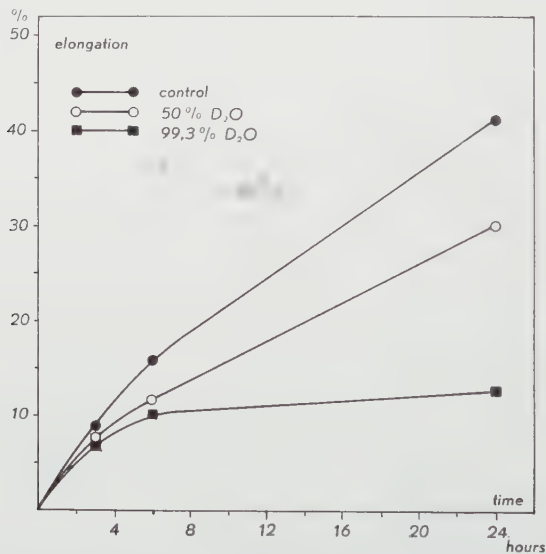


Fig. 4. The effect of D₂O on the autonomous growth of sections.

TABLE 5
The growth of sections in heavy water

	% elongation		
	3 hours	6 hours	24 hours
Control	8,9	15,8	41,1
50 % D ₂ O.	7,6	11,6	30,2
100 % D ₂ O	6,8	10,0	12,6

For the permeability experiments it is important to know the effect of heavy water during the first hours after addition. The experiment described above is not conclusive in this respect. Therefore other experiments were carried out with 50 % D_2O only. This time the heavy water had not been redistilled. The sections were partly placed in heavy water immediately after cutting (fig. 5A), partly after a period of 5 hours in distilled water (fig. 5B). Measurements were made at short time intervals after the immersion in D_2O .

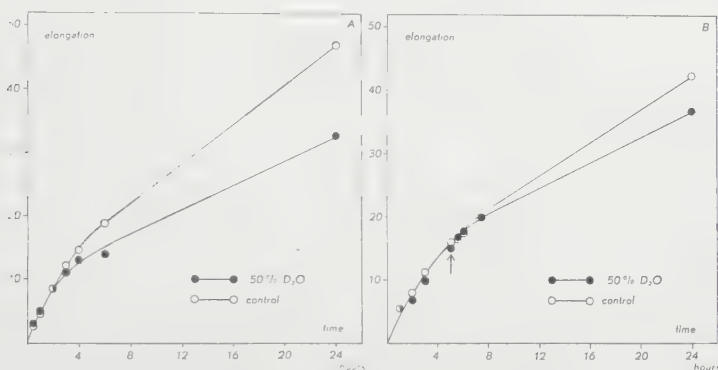


Fig. 5. The effect of 50 % D_2O on the autonomous growth of sections. A. Sections immersed in D_2O immediately after cutting. B. The arrow indicates the moment of addition of D_2O .

The typical experiment shown in fig. 5 again indicates an inhibiting effect, but this does not become evident before 3 or 4 hours after the immersion in D_2O . The final growth rate in D_2O is the same in both cases. For the permeability determinations we may neglect the injurious influence of D_2O , because the sections are in contact with D_2O for only 1 hour, while the damaging effect is not visible until 3 hours have passed after the addition of D_2O .

D. EXPERIMENTS

1. *The effect of pH on the permeability to heavy water.*

The effect of pH was investigated by treating sections with a buffer of a certain pH for some hours before determining the permeability. The time of treatment was varied so as to obtain a maximum effect. The permeability determinations were always carried out with the appropriate buffer mixture in the diffusion vessel. When the time of treatment is given as 0 hours, this means that the sections were not in contact with the buffer before they were placed in the diffusion vessel, which was then filled with a buffer of the stated pH.

Table 6 shows that no effect of the pH was found when the time of treatment was short (0–1 hours), while long periods of treatment with a low pH seemed to increase the permeability to heavy water. In table 6 a measure for the rate of diffusion of D_2O was introduced,

t_i , which is defined as the time taken by the concentration difference to fall to half its value. A decreasing t_i denotes an increasing permeability.

TABLE 6

The influence of the time of treatment on the effect of pH on the diffusion of D_2O

Exp. no.	Time of treatment hours	t_i min.		
		pH		distilled water
		4,17	5,8	
1	0	3,25		3,6
			3,4	3,4
2	1	4,75		4,0
	6	2,25		4,25
		2,25		
3	$\frac{1}{2}$	4,38		
	6	2,75		

A series of experiments was carried out in which the sections were treated with a buffer for 6 hours. Some complete diffusion curves, resulting from these experiments are shown in fig. 3 (page 404), while the t_i values found are brought together in fig. 6. The trend line drawn through the points in fig. 6 must be considered as an arbitrary

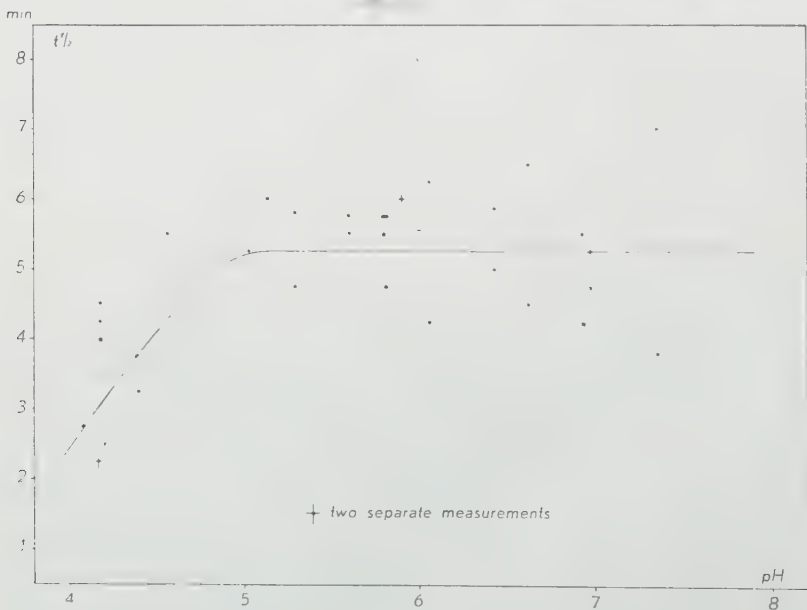


Fig. 6. The influence of pH on the permeability to heavy water.

approximation, for in the figure data are shown obtained at different days and at different times of the year. The variability of the reactions of coleoptile sections from one day to another is too well known to need any further emphasis. Therefore the values shown are not quite comparable.

Twenty different values of the pH between 4.08 and 7.36 have been tried. It is evident from fig. 6, that between pH 5.0 and 7.3 the permeability to heavy water remains constant, while it is definitely increased between 4.0 and 4.5. Compared with pH 6.9 for example generally 60–100 % increase is found. The exact position between 4.5 and 5.0 has not been established. This is very difficult because the small differences in this region cannot be measured significantly.

Three different causes can be held responsible for the modification of the permeability:

1. the difference of pH
2. the difference in the ionic composition of the solution.
3. the growth differences resulting from the different pH values.

1 and 2 cannot easily be separated, because pH and ionic composition change simultaneously. Growth differences, however, may be the cause of the differences in permeability, for a low pH stimulates the autonomous growth of the sections. This is shown in table 7.

TABLE 7
The effect of pH on the autonomous growth of coleoptile sections

pH	% elongation after			
	2 hours	4 hours	6 hours	24 hours
4.08	10.7	14.3	17.9	26.0
4.18	14.6	17.0	19.7	26.8
4.57	6.6	10.3	13.1	22.0
4.93	7.0	11.4	15.7	25.4
5.15	4.6	8.2	10.7	17.0
5.91	3.5	5.8	8.4	18.4

It appears from the table that during the 6 hours of treatment with the buffer mixtures a considerable amount of growth has taken place. Taking two extremes, pH 4.08 and 5.91, growth is twice as much in the former than in the latter. Assuming a constant diameter of the sections (this is approximately true, the increase in diameter being about 3 %), the increase in area will also show the proportion 2 : 1. The total area of sections treated for 6 hours with pH 4.08 is 1.09 times the area of the sections kept at pH 5.91. Nothing is known about the relation between elongation and a secondary increase in permeability, caused through this elongation, but even if it existed it does not seem likely that a difference in permeability of 60–100 % will be caused through a difference in area of only 9 %. Therefore, we are inclined to consider the difference found as an effect of the pH or the changed ionic composition of the buffer mixture.

2. The effect of I.A.A. on the permeability to heavy water

The influence of I.A.A. was investigated in the same way as the effect of the pH in the preceding section. Three different times of treatment were chosen: 0, 1 and 6 hours. The diffusion vessels always contained I.A.A. except in the case of the control determinations. As the optimum growth is found at a concentration of about 1 mg/l I.A.A., only this concentration was used in these experiments. The results are shown in table 8.

TABLE 8

The influence of 1 mg/l I.A.A. on the halving time ($t_{\frac{1}{2}}$) of D_2O diffusion from coleoptile sections

Treat- ment	$t_{\frac{1}{2}}$ min.										
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5 *	Exp. 6 *		Exp. 7 *		Average	
Control	3,7	4,4	7,4	4,0	3,9	4,6	5,4		4,2	4,5	4,68
0 hours				4,0							4,00
1 hours	3,7	5,8	5,4	6,5							5,35
6 hours	4,6		5,4	5,4	5,2		5,2	4,8	5,1		5,10

* I.A.A. added in presence of 1/100 mol. KH_2PO_4 . (pH 4.5)

No opening effect of I.A.A. on the ectoplasm was found in these experiments. With the exception of exp. 3 and 6 I.A.A. appeared to decrease the permeability to heavy water, as an increase in $t_{\frac{1}{2}}$ was found. The exception of exp. 3 may be left out of consideration, because the extremely high $t_{\frac{1}{2}}$ of the control was probably caused by some experimental error.

During the treatment with I.A.A. a considerable amount of growth took place. The control sections grew about 18 %, the sections treated with I.A.A. 35 %. This did not cause an increase in the permeability of the sections which showed the greatest elongation. During the permeability determinations some growth also took place, but the reduced weight remained constant within the experimental error. This could be expected, because the sections absorbed water only. When sections are observed for periods, much longer than 1 hour, a decrease of the reduced weight can be expected as a result of the loss of dry substance, which is used in respiration.

In contradiction with the findings reported in the preceding section it was found that the effect of I.A.A. was the same after 1 and 6 hours treatment. In both cases a slightly decreased permeability was found.

E. DISCUSSION

The experimental results described show very clearly that an increase in the permeability of the ectoplasm cannot initiate growth. The action of pH and growth substances will be localized somewhere else in the protoplasm. In respect to the action of I.A.A. these findings do not imply that the primary action of I.A.A. on the protoplasm is not a physico-chemical effect. As suggested by ANKER (1953), I.A.A. may affect lipophilic membranes in the inner part of the protoplasm.

Our experiments support to some degree a physico-chemical action of I.A.A., because they show that I.A.A. exerts an influence on membranes.

The results are in agreement with the findings of KONINGSBERGER et al. (1947), who also found a decrease in the permeability after the addition of I.A.A. They are at variance with the results of BUFFEL (1949, 1952), who found an opening effect on the ectoplasm. This difference may be caused through a difference in age and treatment of the sections.

The time relationships of the action of I.A.A. seem to differ from those found by KONINGSBERGER et al. (1947), as no effect was found when the I.A.A. was added in the diffusion vessel only (0 hours treatment). In this respect our own data are insufficient, but BUFFEL (1949, 1952) and POHL (1948) also reported a treatment of at least about 1 hour to obtain any influence of I.A.A. This difference may be caused through a difference in material, KONINGSBERGER using isolated protoplasts, the other authors coleoptile sections.

The results of the experiments on the effect of pH on the permeability to heavy water are at variance with the data known in the literature. SEEMANN (1949), for example reports an optimum permeability at pH 6-7. Our data do not show an optimum at all. An explanation of this fact cannot be given yet. It may be a consequence of the difference in methods or material.

The increase in the permeability to water being excluded as cause of the higher growth rate under the influence of I.A.A., the osmotic properties of coleoptile sections will be examined in the next chapter.

CHAPTER III

THE OSMOTIC PROPERTIES

1. INTRODUCTION

It has been stated that a positive suction force must be maintained during growth. Therefore, the effect of growth substances may appear as an increase in suction force. As only a few data about the gross suction force of growing tissues under the influence of growth substances are available, it will be worthwhile to carry out determinations of the suction force. An enhancement of the suction force can be attained in two ways: a rise of the osmotic value of the cell contents, or a lessening of the wall pressure. A third possibility is the existence of an active water absorption, which may be promoted through growth substances. The following formula can be given for the suction force:

$$S = O + A - W$$

in which S means suction force, O osmotic value, A active water absorption and W wall pressure. S and O can be measured directly and this will be done in the experiments to be described in this chapter.

The object of these experiments is to examine whether the suction

force is enhanced through I.A.A. and to what extent changes of the osmotic pressure play a role in the reaction of coleoptile sections on I.A.A. Coleoptile sections are the preferable material for this purpose, because they react on growth substances immediately and grow in distilled water without any external supply of osmotic material, which simplifies the experimental conditions and the interpretation of the results.

Some definitions of the different terms used in this chapter run as follows:

The suction force is the force, expressed in atmospheres, with which water is attracted into a cell placed in distilled water. It is abbreviated as S .

The osmotic value is in numerical value equal to the potential maximum hydrostatic pressure which will develop in a solution if it is permitted to come to equilibrium with pure water in an ideal osmotic system (MEYER, 1945). The osmotic value is expressed in atmospheres and abbreviated as O .

The wall pressure is an inwardly directed hydrostatic pressure, expressed in atmospheres, originating as a reaction of the elastically stretched cell wall. It is abbreviated as W .

The turgor pressure is the outwardly directed hydrostatic pressure of the cell contents on the cell wall. It is abbreviated as T and also expressed in atmospheres. In tissues, T and W are not necessarily equal, as tissue tension or other external pressures may exist which help to counterbalance the turgor pressure. In this case turgor pressure is equal to wall pressure plus external pressure (P):

$$T = W + P$$

2. LITERATURE

The data, which can be gathered from the relevant literature, show that generally the osmotic value of growing tissues remains constant, or decreases only slightly, when a source of osmotic active material is present (e.g. the seed or salts or sugars in the external solution). The amount of osmotically active material per cell increases markedly. These phenomena have been investigated extensively by their discoverer PFEFFER (1893) and by URSPRUNG and BLUM (1924). The latter also measured the suction force in the roots of *Vicia Faba* and found that the suction force is maximum in the zone of maximum elongation. An extensive review of the older literature was given by HEYN (1931, 1940).

Fewer data are available about the influence of growth substances on S and O . RUGE (1937) found a drop in the osmotic value of I.A.A.-treated *Helianthus* hypocotyls. DIEHL et al. (1939) found that the osmotic value in epidermal cells of *Helianthus* hypocotyls treated with I.A.A. was smaller than in the control series. They used, like RUGE did, hypocotyls still attached to the seeds and with a developed root system.

BURSTRÖM (1942) found an increase in osmotic active substances induced by I.A.A., while the osmotic value during growth was dependent on the nutritional condition of the roots.

OVERBEEK (1944), LEVITT (1948) and HACKETT (1952) showed that the osmotic value of potato tuber discs is decreased after I.A.A. treatment (see Ch. I, par. 2).

Data about the influence of growth substances on the suction force are very scarce. RUGE (1937) stated that S decreased after treatment of hypocotyles with I.A.A., while BRAUNER and HASMAN (1949) found that the suction force of I.A.A.-treated potato tuber discs has fallen below that of discs kept in water after 6 hours exposure. After longer exposure times, however, the suction force is greater in the treated discs, in spite of the greater amount of water absorbed. The differences are very small, however.

So far it seems to have been established that I.A.A. does not enhance the osmotic value, while the data about the suction force are conflicting. Moreover, a drawback of these investigations is that generally, S and O have not been determined for the same material and in the same experiment.

3. METHODS

The "simplified method" of URSPRUNG and BLUM (1923) allows the determination of the suction force by measuring changes of length in solutions with different osmotic values. For the determination of the osmotic value the external concentration at which incipient plasmolysis occurs must be known. Incipient plasmolysis is very hard to observe in tissues. However, when tissues are placed in solutions with higher osmotic values than those used in the suction force determinations maximum shrinkage will occur at a certain osmotic value of the external solution. In other words, the length of the tissues becomes constant within the experimental variation, independently of the hypertonic external osmotic value. This means that the tissue is plasmolysed and has no turgor. The first concentration, which gives the constant length, can be considered as the concentration of incipient plasmolysis. Of course this is an approximation, which will be the more accurate, as the concentration differences employed are smaller. When the initial length and the length at incipient plasmolysis are known, the osmotic value can be calculated.

The above principle has been applied in this investigation as follows: 10 sections, prepared as described on page 390, were mounted on a slide and placed in a Petri dish. A number of parallel series was prepared. As osmotic active agent mannitol was chosen, because it permeates very slowly and does not support growth. Different molar concentrations were prepared and each series was measured and placed in a different concentration. 50 ml solution was used for 10 sections. The concentrations varied between 0 and 1,0 mol. in 1000 grams H_2O . After 1 hour the sections were measured again and the changes of length were calculated. The values found are

brought together in a graph, which shows the change in length as a function of the change in concentration or osmotic value of the external solution. The point of intersection of the graph and the abscissa shows the value of the suction force, while the point at which the graph becomes horizontal (always below the abscissa) denotes the concentration of incipient plasmolysis. This method is referred to as method I.

The results obtained with the coleoptile sections give a crude approximation of the order of magnitude, as it is not possible to obtain reliable results with concentration differences smaller than 0,05 mol. This is a difference in osmotic value of about 1,1 atm. Smaller differences cannot be employed due to the variation in reaction between the sections.

Of course it is possible to determine suction force and osmotic value after various times of treatment with growth substances. But the number of treatments to be tested in one experiment is limited, as at least 8 to 10 series of ten sections are needed for every determination of suction force, plus osmotic value. It will be found desirable to use 11 or 12 series, which allow the use of more different concentrations of the mannitol solutions with small grades, especially near the expected values of S and O_i (O at incipient plasmolysis). In addition to the treated series a control series must be run, so that in all, at least 20 series of 10 sections must be prepared. This limits the number of treatments which can be examined in one experiment. For the determination of the suction force 5 series is the minimum number.

Instead of measuring the differences in length, it is possible to determine the changes in water content in solutions with a different osmotic value by weighing the sections before placing them in the solutions, and after one hour's submersion. This method is referred to as method II. It allows the determination of the suction force only, as it is not possible to determine the point of incipient plasmolysis in this way. The results are somewhat more exact than those obtained by measuring the changes of length.

4. THE EFFECT OF I.A.A. ON THE OSMOTIC PROPERTIES

To start with, S and O were determined immediately after cutting the sections and after some hours' submersion in distilled water. One

TABLE 9

Suction force, osmotic value and wall pressure of coleoptile sections immediately after cutting and after some hours in distilled water

Exp. no	Time of treatment in dist. water hours	S atm.	O atm.	W atm.
1	0	8,63	—	—
2	0	6,93	12,72	5,79
3	5	2,60	10,50	7,90
4	6½	3,58	10,62	7,04
5	5½	2,65	12,78	10,13

of these experiments is presented in fig. 8, while the data are brought together in table 9.

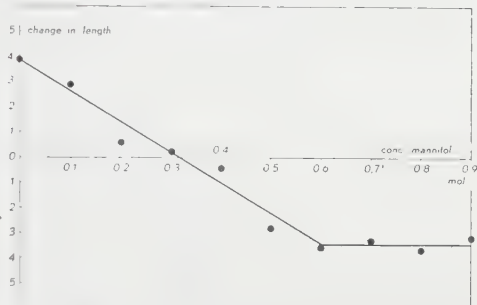


Fig. 7. The changes in length of sections immediately after cutting immersed in different concentrations of mannitol.

It appears from the table that S is very high, 7–8 atm., immediately after cutting and drops rather fast in distilled water. Assuming that no active absorption of water occurs (this will be discussed later)

$$S = O - A - W$$

is simplified to

$$S = O - W$$

From this W can be calculated. The results are compiled in the last column of table 9. W appears to be fairly high in the coleoptile and shows a tendency to increase during the immersion of the sections in distilled water.

After establishing these facts the suction force of 7½-mm sections was determined by using method II. The sections were treated with

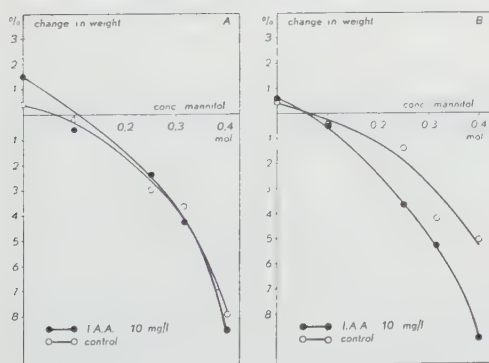


Fig. 8. The effect of I.A.A. on the suction force. A. After 3½ hours treatment with I.A.A. B. After 27½ hours treatment with I.A.A.

I.A.A. before the determinations were carried out. The results are shown in table 10, while experiment 3 is presented in fig. 8. From these results it is clear that S must be increased by I.A.A., while the increase is not found after a long time of treatment.

To examine the osmotic value a more extensive series of experiments was carried out by using method I. Before determining S and O the sections were treated with I.A.A. 5 hours was chosen as the most

appropriate time of treatment. The results are given in table 11 and a typical experiment is shown in fig. 9. Table 11 shows that after 5 hours of treatment with I.A.A. the suction force is not greater evidently than in the control series. Considering the experiments 1

TABLE 10
The effect of 10 mg/l I.A.A. on the suction force of 7½ mm coleoptile sections

Exp. no.	Treatment	$S_{I.A.A.}$ atm.	$S_{Contr.}$ atm.
1	5 hrs. dist. H ₂ O, 1 hr. I.A.A.	2,71	1,58
2	2½ hrs. I.A.A.	4,07	3,36
3	24 hrs. dist. H ₂ O, 3½ hrs. I.A.A.	2,46	1,34
	24 hrs. dist. H ₂ O, 27½ hrs. I.A.A.	1,12	1,12

up to and including 8 and leaving the differences in I.A.A. concentration out of account, it appears that $S_{I.A.A.}$ and $S_{contr.}$ are the same in 6 cases, $S_{I.A.A.}$ is greater in one case and smaller in 3 cases (differences smaller than 0,5 atm. have been neglected). After shorter times of

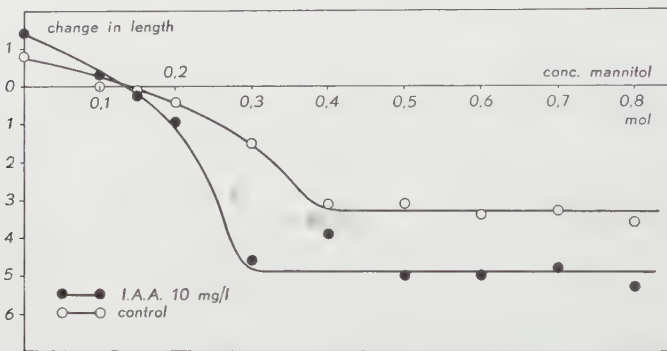


Fig. 9. The effect of 5 hours treatment with 10 mg/l I.A.A. on the suction force and the osmotic value at incipient plasmolysis. The change in length is given in scale units of the eyepiece micrometer used in the measurements.

treatment, however, $S_{I.A.A.}$ is nearly always higher than $S_{contr.}$ (compare table 10). Fig. 12 (page 427) shows that the growth rate is maximum during the first hours after the addition of I.A.A. After 5 hours nearly half of the total elongation to be found after 24 hours has taken place. The growth rate of I.A.A.-treated sections is still higher than that of the control sections, but much lower than immediately after the addition of I.A.A. This may offer an explanation for the negative results obtained after prolonged treatment with I.A.A. The fact that no significant increase in S is found after 5 hours does not exclude definitely the existence of an increase. The method used is not very accurate and small differences will escape from observation.

The results of the determinations of O give more clear-cut results. In all experiments $O_{I.A.A.}$ is lower than $O_{contr.}$. This is in accordance with the data reported in the survey of the relevant literature: The

possibility of osmotic material being formed in the sections through hydrolysis of higher carbohydrates (starch being the most likely source) cannot be rejected with the argument that the osmotic value does not increase. Only direct determinations of the total amount of sap solutes, or a determination of the product of osmotic value and volume (VAN 'T HOFF's law), can give information about this

TABLE 11

The effect of I.A.A. on suction force and osmotic value. I.A.A. was added immediately after cutting

Exp. no.	Time of treatment hours	Conc. I.A.A. mg/l	S atm.		O atm.		W—A atm.	
			I.A.A.	Contr.	I.A.A.	Contr.	I.A.A.	Contr.
1	5	10	1,15	2,33	6,70	9,63	5,55	7,30
2	5	10	1,90	2,20	6,04	9,60	4,14	7,40
3	5	10	2,56	1,40	7,32	7,46	4,76	6,06
4	5	12,2	2,31	2,19	6,01	8,44	3,70	6,25
5	5	10	2,84	2,94	6,08	8,41	3,24	5,47
6	5	10	2,45	3,31				
		10	2,70		8,12		5,42	
7	5			2,50		8,42		5,92
		1	2,60		8,19		5,59	
8	5	1	2,63	2,51	8,21	8,57	5,58	6,06
† 9	5	1	1,87	2,60	7,26	7,51	5,39	4,91
		1	2,20					
*10	1			2,20				
		10	2,70					
*11	1	10	4,20	2,82				

† The control sections showed a higher growth rate after the determinations of *S* and *O* than the I.A.A. series.

* The sections were kept in distilled water for 5 hours after cutting, then I.A.A. was added.

problem. If no osmotically active material is formed the product mentioned must be approximately the same for control and for I.A.A.-treated sections. If, on the other hand, I.A.A. causes a stimulation of the formation of osmotically active substances the product must be higher in the presence of I.A.A. The available experimental data for the experiments listed in table 11 allowed an approximate calculation of the product. Table 12 shows the values of the product found in a number of experiments. As the diameter of the sections is nearly constant, the length of the sections was substituted for their volume. The results of the calculations are given in table 12. The product $O \times L$ shows a tendency to decrease under influence of I.A.A. This means that I.A.A. has no effect on the formation of osmotic material. Hydrolysis of starch may be promoted by I.A.A., but in that case the resulting products are entirely consumed in the metabolism.

From the values found for the osmotic value it can also be concluded that, although the osmotic value of the I.A.A.-treated sections is smaller, both I.A.A.-treated and control sections have about the

TABLE 12

The effect of 5 hours treatment with 10 mg/l I.A.A. on the product osmotic value $\times$ length

Exp. no.	$O \times L$	
	I.A.A.	Control
1	349	432
2	316	426
3	362	326
4	312	371
5	313	376
7	397	381

same suction force. This indicates that I.A.A. affects another component of the suction force. As no proof is available that an active absorption of water promoted by I.A.A. does not exist, this possibility must be taken into account. The formula denoting the suction force was stated to be

$$S = O + A - W$$

This can be written as

$$O - S = W - A$$

As O and S are known, the difference $W - A$ can be calculated. The calculated values can be seen in table 11. Indole-3-acetic acid decreases the difference $W - A$. Thus W or A may be changed, W being decreased or A being increased. A choice between these two possibilities cannot be made, but an indication may be found in fig. 9 (page 416), which shows that I.A.A.-treated sections shrink more during plasmolysis than do the control sections.¹ Consequently, the treated sections are stretched elastically to a greater extent in turgescence condition, which implies a greater elasticity of the cell wall, as the sections immersed in water are always fully turgescence. Thus I.A.A. may influence the wall properties and in this way change the suction force.

5. DISCUSSION

Although no evident change in the magnitude measured as the suction force S has been found after longer times of exposure to I.A.A., probably because of methodical limitations, no doubt is left

¹ In fig. 9 the changes in length are given after the immersion of the sections in mannitol solutions. The initial length (before the immersion in mannitol) will be greater in the I.A.A.-treated sections than in the control sections, for I.A.A. will have promoted elongation. In the experiment shown in fig. 9 the above-mentioned initial length of the control sections was 44,2 units and that of the I.A.A.-treated sections was 52,1 units. The length immediately after cutting the sections was 39,8 and 39,7 respectively. The elastic extension was 3,3 and 4,9 respectively. These data demonstrate that the irreversible increase in length is greater than the increase in the reversible part of cell length after the addition of I.A.A.

about the fact that growth substances affect the suction force. It has been proved that the osmotic value of the cell contents decreases with increasing elongation, while the suction force remains more or less constant. The osmotic value is lowest in sections treated with I.A.A. and the value of the product $O \times L$ indicates that I.A.A. does not increase the total quantity of sap solutes. But even if the osmotic value remains constant during the elongation, this cannot be an explanation for the higher growth rate, which is maintained in I.A.A., for the wall pressure would increase very quickly and would prevent any further growth. The same argument is valid for the possible effect of I.A.A. on an active process of water absorption.

Therefore, a change in wall properties seems to be necessary, the more so as both plastic and elastic stretching of coleoptile tissue are rather limited. A change in the elastic properties of the wall was indicated in the reported experiments, as has been pointed out on page 418. This is a fairly direct proof. More indirect evidence can be found in the experiments of table 10. In fig. 8 it focusses the attention on the fact that both in figure A and figure B the graph representing the changes of weight of the I.A.A.-treated sections is less concave towards the abscissa than the one representing the control. HASMAN (1943) and BRAUNER and HASMAN (1949) pointed out that the positive part of the *S*-curve (above the abscissa) will be less concave as the tensility of the cell wall is higher. These experiments support therefore an effect of I.A.A. on the cell wall. This will be investigated more in detail in experiments to be described in the next chapter.

It must be emphasized that the suction force that is found after prolonged immersion in distilled water or in I.A.A., originates from growth and that it cannot be accounted for osmotically. This fact should be well borne in mind. A simple calculation in using the formula $S = O - W$ will not give any information about the origin of this suction force, since the unknown active component resulting from growth always plays a part.

An estimate of the magnitude of this active component is possible, when it is assumed that the sections are fully turgescient after some hours' (3–5 hours) immersion in distilled water. This assumption is justified by the fact that in osmotic studies it takes fairly short times to establish an equilibrium. If the assumption is correct the wall pressure will be equal to the osmotic value in numerical value. In that case the active component of the suction force will be equal to the suction force found. It is therefore likely that the suction force measured after prolonged treatment with distilled water or with an I.A.A. solution must be ascribed to growth alone.

CHAPTER IV

THE ELASTIC PROPERTIES OF THE CELL WALL

1. INTRODUCTION

The experimental results described in the preceding chapter suggested an effect of I.A.A. on the elastic properties of the cell wall. The experiments to be reported here were carried out to provide this hypothesis with more evidence, in order to establish the time relationship of the possible effect of I.A.A. on the wall and to compare the effect of I.A.A. on the cell wall with its effect on the growth rate. First a survey of the literature on the subject will be given.

2. LITERATURE

In view of the great number of publications available it is not possible to give a complete review in the limited space available. This survey will therefore be restricted to the effect of growth substances on the elastic properties of the cell wall.

HORREUS DE HAAS (1929) suggested a relation between growth substances and wall properties. He found that the elasticity is higher in the upper side of geotropically stimulated roots of *Vicia Faba* than in the lower side. Decapitated coleoptiles were less elastic than intact coleoptiles.

HEYN (1931) made extensive investigations into the effect of decapitation on the wall properties of *Avena* coleoptiles. Decapitation lowered elastic and plastic extensibility, while the extensibility increased again after the application of growth substance. HEYN concluded that the elasticity has no importance at all for growth but that it is increased as a result of elongation. Growth substances would regulate growth via the plasticity of the cell wall.

HEYN and VAN OVERBEEK (1931) determined the plastic and elastic extensibility of coleoptile sections. Both are increased by growth substances. The increase in elastic extensibility is rather considerable.

SÖDING (1931, 1932, 1934) found that elastic and plastic extensibility and growth rate are interconnected and also show a relation with the amount of growth substance available. The turgor extension is increased after treatment of *Avena* coleoptiles with growth substance for $1\frac{1}{2}$ hour. SÖDING considered the increase in both plastic and elastic extensibility as a result of the elongation and as an accompanying phenomenon.

RUGE (1937) found that the elastic extension of *Helianthus* hypocotyls was increased after 18-24 hours treatment with concentrated I.A.A. pastes. RUGE postulated that this increase originated as a result of the decrease in wall thickness during elongation. The author states that the wall properties of plasmolysed and dead cells are also changed by I.A.A. His experiments, however, are liable to critical consideration (see page 430).

DIEHL et al. (1939) treated *Helianthus* hypocotyls with lanoline

pastes containing I.A.A. They found a maximum elastic extensibility after 2 hours treatment. The wall properties were changed after 1 hour treatment with I.A.A., the change is maximum after 2 hours and is maintained for some hours.

GESSNER (1936) observed that during phototropic stimulation the shadow side showed an extremely high extensibility. Light decreased both elastic and plastic extensibility in *Helianthus* hypocotyls. The author ascribes these changes to assumed changes in the concentration of the growth substances in the tissue after illumination.

AMLONG (1936) found that the roots of *Vicia Faba* grew faster after decapitation, but the elastic and plastic extensibility were smaller after decapitation. This implies that there is no direct relation between wall properties and growth rate. The different concentration for growth substance action on roots and stems cannot be explained through an effect on the cell membrane. AMLONG (1939) found that low concentrations of growth substances promoted growth rate, plastic and elastic extensibility. The optimum concentration is the same for all three.

The results of BURSTRÖM (1942) are partly at variance with those of AMLONG, as BURSTRÖM found that inhibiting concentrations of I.A.A. still increased the elastic extension and no joint optimum could be established. BURSTRÖM states that the increase in extension precedes elongation and is important as an initiator of growth. FREY WYSSLING (1948, 1952) used some of BURSTRÖM's data in calculations and obtained the result that the Young's Modulus decreases most markedly during the early phase of elongation, but rises again steeply without an obvious decrease of the growth rate. The author considers the increased elasticity as an essential part of the elongation process.

From these results it appears that, generally speaking, growth substances effect a rise in the elastic properties of the cell wall, determined as elastic extension or as elastic extensibility. Most of these authors reject the idea that the elastic properties occupy a key position in the elongation process, while some of them assume that an increased elasticity is important in the starting phase of growth.

3. METHODS

Sections were prepared according to the procedure described on page 390. These sections were treated with I.A.A. under the usual conditions for the section test (page 390). After some time the length of the sections was recorded and they were removed from the solution containing I.A.A. and plasmolysed with 1 mol. mannitol (this means 1 mol. mannitol solved in 1000 grams water). After 1 hour in the mannitol solution the sections were measured again. The difference between the length of the turgescient cells (L_t) and the plasmolysed cell (L_p) was calculated. This difference (dL) was called elastic extension. Thus:

$$dL = L_t - L_p$$

A change in elastic extension was regarded as an indication of a change in the properties of the cell wall. The extension was noted in scale units of the eyepiece micrometer used in the measurements. One unit corresponds to 60μ .

To attain a relation with the length of the sections we introduced a factor E , which was defined as:

$$E = dL/L_p \times 100.$$

The comparison of E -values comes to the comparison of the average elasticity of the corresponding sections. For a discussion of E see page 439.

4. THE INFLUENCE OF I.A.A. ON THE ELASTIC EXTENSION

In a series of experiments sections were treated with I.A.A. for about 5 hours. The I.A.A. was added immediately after cutting. The elastic extension was determined and compared with the extension of a control series. The results are brought together in table 13.

TABLE 13
Elastic extension after 5 hours treatment with I.A.A.

Exp. no.	Elastic extension scale units		E %		Number of series and of sections		Conc. I.A.A. mg/l
	I.A.A.	Control	I.A.A.	Control	I.A.A.	Control	
1	5,6	3,4	12,0	8,3	9×10	6×10	10
2	5,3	3,5	11,3	8,6	7×10	5×10	10
3	5,2	3,3	11,9	8,0	4×10	4×10	10
4	3,7	2,5	8,1	6,1	5×10	5×10	10
5	4,4	3,2	9,2	7,7	6×10	4×10	12,2
6	4,8	3,3	10,3	8,1	6×10	5×10	10
7	6,6	3,6	13,4	8,5	1×10	1×10	10
8	5,3	2,7	10,7	6,1	2×10	2×10	1
9	4,8	3,3	9,3	8,0	4×10	4×10	1
10	4,2	2,2	8,5	5,2	5×10	5×10	1

The table shows that in all these experiments the elastic extension is higher after treatment with I.A.A. This increased extension indicates very clearly that the final effect of I.A.A. is found in the cell wall, but it does not prove that the primary reaction of the growth substance is also located in the wall. It can only be said that in general fast growing cells have a greater elastic extension than slowly growing sections.

The concentrations used in these experiments all have a promoting influence. The increase in elastic extension is for both concentrations in the same order of magnitude. From other experiments it may be concluded that in one and the same experiment 10 mg/l I.A.A. has a greater effect than 1 mg/l. A more systematic investigation of this phenomenon has not been made.

All determinations mentioned in table 13 were carried out after about 5 hours treatment with I.A.A. For this reason it seemed desirable

to investigate the time sequence of the increase in elastic extension and to compare this with the growth reaction. This was accomplished by adding I.A.A. to a number of series and determining the elastic extension at different times after immersion in the solutions. Table 14 shows results with sections which were treated immediately after cutting. After 5 hours treatment the increase in elastic extension is

TABLE 14
Elastic extension in relation to the duration of the treatment with I.A.A.¹

Time of treatment hours	Elastic extension scale units		E %		Conc. I.A.A. mg/l
	I.A.A.	Control	I.A.A.	Control	
0		4,4		11,8	10
1	5,4	5,0	13,7	13,0	
5	7,4	2,9	14,4	6,8	
24	6,7	2,8	11,6	6,1	

¹ Every value was determined from 1 series with 10 sections

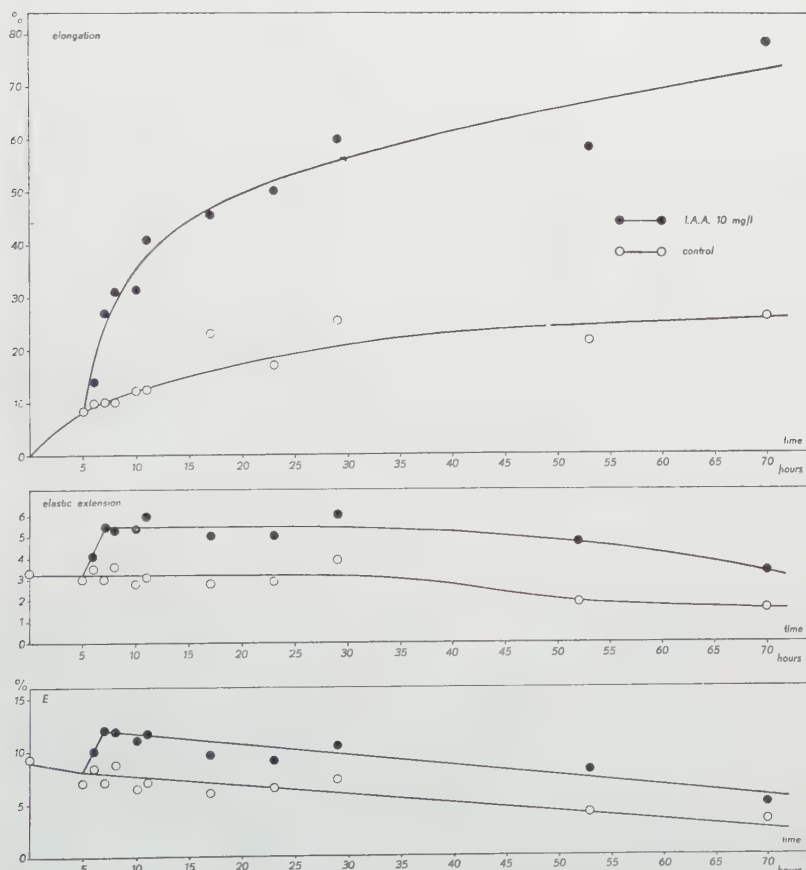


Fig. 10. The effect of 10 mg/l I.A.A. on elongation, elastic extension and E .

considerable, especially when one compares the absolute values. After 1 hour, however, there is no significant difference with the control.

This problem was investigated more extensively with sections treated with I.A.A. after immersion in distilled water for 5 hours after cutting. The results are collected in table 15.

The experiments 3 and 5 indicate that the first 2 hours are very

TABLE 15

Elastic extension in relation to the time of treatment with indole-3-acetic acid. The sections were immersed in distilled water during 5 hours before treatment

Exp. no.	Time of treatment hours	Elastic extension scale units		E %		Conc. I.A.A. mg/l
		I.A.A.	Control	I.A.A.	Control	
1	0		3,1		7,5	10
	$\frac{1}{4}$	3,0	3,0	7,2	6,9	
	$\frac{1}{2}$	3,5	3,5	8,7	8,5	
	1	3,8	3,3	8,9	8,3	
	$1\frac{1}{2}$	4,8	3,0	11,5	7,3	
	2	5,1	3,0	12,1	7,2	
2	0		3,0		7,0	10
	1	4,1	3,5	9,7	8,6	
	2	5,5	3,0	11,9	7,1	
	3	5,3	3,6	11,8	8,7	
	5	5,4	2,8	11,0	6,6	
	6	6,0	3,1	11,7	7,1	
	12	5,2	2,8	9,6	5,9	
	18	5,2	2,9	9,1	6,5	
	24	6,1	3,8	10,3	7,8	
	48	4,8	1,9	8,1	4,0	
	65	3,4	1,6	4,8	3,2	
3	0		3,6		8,6	10
	1	4,6	3,7	10,3	9,0	
	2	5,9	2,5	13,1	6,0	
	19	7,2	3,1	11,3	6,5	
	24	6,2	4,3	9,2	8,8	
	41	7,8	2,6	11,0	4,8	
4	0		2,8		6,3	1
	$\frac{1}{2}$	4,0	3,1	9,6	7,3	
	1	4,5	3,3	10,2	7,7	
	18	8,3	3,9	12,7	5,9	
5	0		3,8		9,3	1
	1	4,1	3,7	9,5	9,1	
	2	4,9	3,4	10,9	8,0	
	3	5,2	3,2	10,9	7,7	
	21	6,0	3,1	9,9	6,7	
6	1	4,6	3,7	10,8	8,8	10
7	1	4,6	3,6	10,4	8,3	1

important for the reaction of the elastic extension on the addition of I.A.A. It seems that the maximum value of E is reached after 2 hours, while the extension in absolute measure still increases. Lack of data between 2 and 20 hours after the addition of I.A.A. made these results less valuable. Experiment 2 was therefore carried out (fig. 10). Elongation, extension and E were determined at different times. The fastest growth takes place during the first hours after the addition of the growth substance. The elastic extension reaches its maximal value after 2 hours and remains more or less constant until 24 hours after the immersion in the I.A.A. solution. After 24 hours it diminishes perceptibly. As the extension remains the same and the length of the sections increases, the E -values decrease from the second hour. This is shown in the lower graph in fig. 10.

Not every experiment showed a constant elastic extension after 2 hours. This can be seen from experiment 3 for example. Here the values found after 19 and 41 hours are much higher than the extension after 2 hours. This frequently occurs and will also be found in the experiments described in the next chapter. However, the extension increases more slowly after 2 hours, than from 0 to 2 hours. Generally, this effect will not be found when using E as an indicator. Then a decrease is seen from the second hour.

Another interesting question is: when does the first measurable reaction occur? Experiment 1 shows that it takes at least 1 hour before any reaction can be detected, while the greatest increase appears between 1 and $1\frac{1}{2}$ hours after adding I.A.A. This is also illustrated by data from table 16. For this table the same experiments have been used as in table 15. Sometimes an appreciable promotion of

TABLE 16
Elastic extension after auxin treatment in % of the control

Time hours	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7
$\frac{1}{4}$	100						
$\frac{1}{2}$	100						
1	115	117	125	129 132	111	124	128
$1\frac{1}{2}$	160						
2	170	184	184		149		
3		148			163		

the elastic extension is found after 1 hour, but a much greater effect is always found between 1 and 2 hours after the addition of I.A.A. This is shown very clearly in fig. 11. Here the ratio $E_{\text{I.A.A.}}/E_{\text{contr.}}$ has been used for comparison. The ratios found in this case are somewhat different from those derived from the elastic extension, but the general trend is the same.

It is desirable in view of the above stated results, to examine the growth reaction during the first hours after addition of growth substance. This study might be realized with data from the experiments shown in table 15, for example exp. 2 (fig. 10), a procedure, less

advisable, because in that case every growth rate determination is made on a different series of sections. It may cause great differences in the case of growth measurements at short time intervals. We therefore used for this analysis growth curves of one series of sections (fig. 12).

The broken lines in fig. 12 were determined with sections which were immersed in distilled water before I.A.A. was added. For about

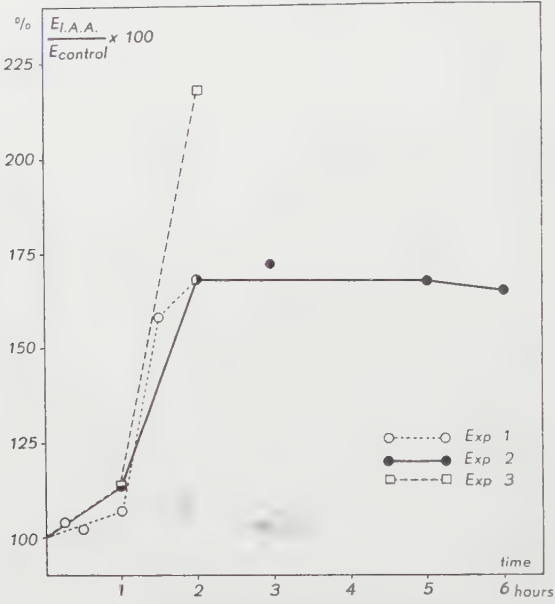


Fig. 11. For explanation see text.

5 hours after the addition of I.A.A. the growth rate will virtually be the same, as is indicated in fig. 12 by the constant slope of the growth curve. Thus about a linear relation exists between time and elongation during this time.

TABLE 17

The growth rate of isolated sections (kept for 5 hours in dist. water first) during the first hours after the addition of I.A.A. SD.: standard deviation

Conc. I.A.A. mg/l	Growth rate % elongation/hour								
	Exp. 1			Exp. 2					
	1st hr.	2nd hr.	3rd hr.	1st hr.	SD.	2nd hr.	SD.	3rd hr.	SD.
0	1,2	0,9	0,9	2,2	1,06	1,4	0,70	1,6	0,52
0,01				3,8	0,92	2,9	0,57	3,1	0,36
0,1				3,9	0,88	2,9	0,57	3,2	0,79
1	7,1	7,8	7,4	2,7	0,68	2,9	1,10		
10	4,9	4,8	4,7						

This is confirmed by the data shown in table 17. Exp. 1 in the table is the same as the one shown in figure 12. The data given in table 17 for exp. 1 have been partly estimated from the growth curves. In both experiments the sections used for the analysis of the growth rate were treated with distilled water for about 5 hours after cutting

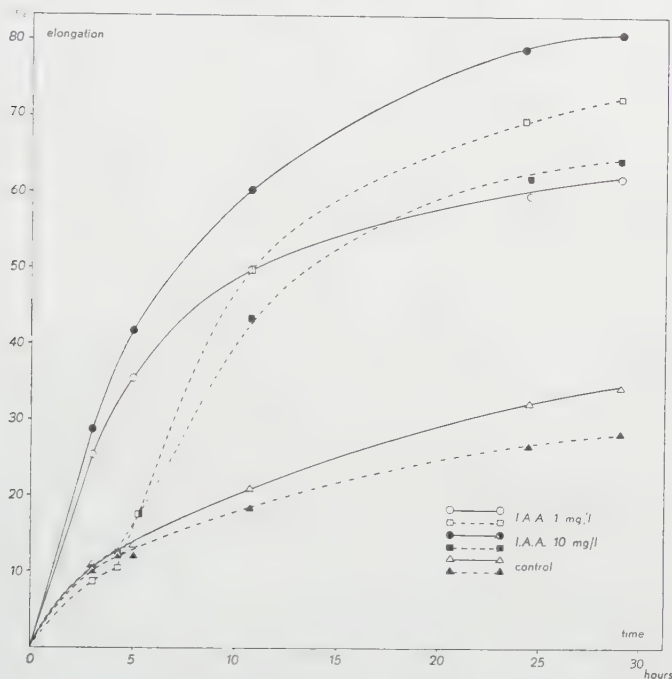


Fig. 12. Growth curves of sections treated with I.A.A. and of control series. The solid lines are determined with sections immersed in I.A.A. immediately after cutting. For the broken lines sections were pretreated with distilled water for $4\frac{1}{2}$ hours.

them before the I.A.A. was added. The table shows that the growth rate remained constant within the limits of the experimental error during 3 hours after the addition of I.A.A. This was also found for different concentrations of I.A.A. The growth rate reaches its maximum value in the first hour, without a visible lag. This behaviour is quite different from the facts found with the elastic extension. There seems to be no parallel between the response of the growth rate and the elastic extension to the external addition of indole-3-acetic acid.

5. DISCUSSION

Growth substance added to isolated sections of the *Avena* coleoptile causes changes in growth rate and in elastic extension. The time sequences of these changes are not identical. The growth rate increases immediately after the addition of I.A.A. and remains constant for

several hours. The results of determinations of the elastic extension seem to indicate a time lag, while the extension does not increase any more — or only very slowly — after 2 hours exposure to I.A.A. This might suggest that no direct relation exists between growth rate and elastic extension.

However, some objections must be considered. In the first place the growth reaction may show a time lag too, but a rather short one, a fact very difficult to discover owing to the variability of the material and the error of the measurements, especially as very small differences are involved. It does not seem very likely, however, for GAST (1942) found that roots of maize seedlings immediately responded to I.A.A., after they had been in contact with the growth substance for only two seconds. RIETSEMA (1950) also found that I.A.A. brings about a rapid increase in growth rate.

Another possibility is that the time lag of the response of the extension is shammed by the limitations of the method. This does not seem very likely either. A minimum change must be effected indeed before it can be measured with the method used. Assuming a constant rate of increase in extension, no increase can be expected during the first measurements, for instance after $\frac{1}{4}$ and $\frac{1}{2}$ hour. If the extension would change at a constant rate the effect after one hour would be about half of the total increase to be found after two hours. This is of a magnitude that might be detected in the section test. If this assumption were true, a rapid increase ought to be found between $\frac{1}{2}$ and 1 hour after the addition of I.A.A. and a proportionally slower increase between 1 and 2 hours. This does not hold true.

For this reason we are inclined to consider the difference in time sequence between elongation and extension as a real and important phenomenon. Apparently the elastic extension is not directly connected with the elongation, but the change in extension may indicate a fast growing of the primary wall without any appreciable apposition of secondary layers (see page 439). In that case the increase in elastic extension is an accompanying phenomenon.

CHAPTER V

THE MECHANISM OF THE ACTION OF GROWTH SUBSTANCES ON THE CELL WALL

1. INTRODUCTION

It appeared from the data given in the preceding chapters that growth substances influence the cell wall. The mechanism of this action has been passed by in silence, so the following part of this investigation is engaged with this problem. In the literature on the subject different opinions have been presented, but a settled explanation is not yet available. This will appear from the selection of the relevant literature in section 2.

The main problem will be to discriminate whether the action of

growth substances is a direct one or whether the changes in wall properties are effected through the intermediary of the protoplasm. Two main ways are open to approach this object:

1. An attempt to destroy the structural relation between protoplasm and cell wall, e.g. by plasmolysing or killing the cells.
2. An attempt to influence the wall properties with substances, which affect protoplasmic activities (growth substances are of course excluded), e.g.
 - a. Substances acting as a metabolic substrate.
 - b. Substances inhibiting the metabolism.

These different possibilities will be tested in this chapter (sections 3, 4 and 5). We may expect that these experiments will also give some information about the processes and paths along which the change is brought about.

2. LITERATURE

As has been stated, many different opinions have been presented about the action of growth substances on the cell wall, some of which will be reviewed here. The greater part of the investigations has been confined to the plasticity of the cell wall, but the problem will be the same, irrespective whether plasticity or elasticity is considered.

HEYN (1940) is inclined to accept a direct action of growth substances on the cell wall and is in this regard at variance with his earlier statements (see below). He bases his opinion on experiments of HEYN and VAN OVERBEEK (1931) who found that the plasticity was changed by growth substances at 4° C. This might exclude an appreciable formation of wall substances, which has been proved later by BONNER (1934). In its turn this fact might imply that the synthesis of wall material is not necessary for growth and that, therefore, growth substances act directly on the cell wall. BONNER's results, however, are not quite convincing, as an effect of growth substances can only be found when the sections are treated with the growth substances for one hour at 25° C, and then removed and placed at a temperature of 2° C. As our own experiments showed (chapter 4), some change took place in the cell wall after 1 hour treatment with I.A.A. Therefore, the alleged difference between the control and the treated series may have its origin in the one hour of treatment at a high temperature. The same objection can be raised against the experiments of HEYN and VAN OVERBEEK (1931). No effect of growth substances exists when the coleoptiles, or sections, are kept at the low temperature from the beginning of the experiment. This was found in some of our own experiments (unpublished) and was also found by BONNER himself (1934). Moreover, considering the rather crude technique used by BONNER, we may expect that the formation of small amounts of wall material will not be detected, while they are sufficient to keep the primary cell wall growing. The bulk of the increase of wall material,

as found by BONNER, will be caused through the apposition of secondary layers. This process might be inhibited by low temperatures.

This hypothesis finds some confirmation in investigations about the wall structure during growth. BONNER (1936) showed that the wall structure remains the same during elongation. He suggests that the change of wall properties is brought about through a loosening of the "Haftpunkte" in the micellar network by the growth substances. This can, of course, be accomplished directly or indirectly. But BONNER is now (1936) of the different opinion that growth cannot be a simple mechanical process. To maintain the structure new material must be woven in. This is a more detailed variant of the theory suggested by SACHS (1874).

BURSTRÖM (1942) assumes that I.A.A. causes an increase of the elasticity through a direct action on the cell wall. Growth, however, would be accompanied by the formation of new cellulose micellae.

RUGE (1937) also defends a direct action of I.A.A. on the cell wall. He explains the loosening of the "Haftpunkte" as a result of the swelling of intermicellar substances under the influence of growth substances. He supports this hypothesis with experiments about the promotion of the swelling of different slime bodies by I.A.A. These experiments were performed with inadequate techniques and too high concentrations of I.A.A. BLANK and DEUEL (1943) examined the effect of I.A.A. on the swelling and the viscosity of wall substances in model experiments. In physiological concentrations they did not find an effect of I.A.A., while in higher concentrations I.A.A. showed a normal acid effect. So they rejected RUGE's hypothesis. RUGE (1942) still maintains a direct effect of growth substances on the cell wall, presuming an influence on the heteropolar bonds between 2 OH-groups of two adjacent cellulose molecules. In view of the results of many other investigators, however, a direct action seems most unlikely.

This is illustrated for example by HEYN's statement (1931) that elongation cannot be resumed after plasmolysis. This phenomenon has been found by REINHARDT (1899) for roots of different plants after plasmolysis in sucrose solutions. It can be considered as an indication for an interaction between the protoplasm and the cell wall in the process of elongation.

GESSNER (1934) found that the factors which affect the wall properties do not act when the cell has been plasmolysed. Thus the protoplasm will play an important role.

THIMANN and BONNER (1933) could not establish a direct stoichiometric relationship between the amount of growth substance entering the cell wall constituents on which it acts. They also calculated that the quantities of growth substance involved in elongation are not sufficient to form a monomolecular layer over the cell wall. These authors suggested an indirect action of growth substances on the cell wall. Their conclusions seem to be justified.

KONINGSBERGER (1947) pointed out that, assuming a direct swelling effect of growth substances on the cell wall, it is not possible to explain

the optimum curve found. In that case one would expect a sigmoid saturation curve.

SÖDING (1934, 1952) considers the intussusception of new wall material as the most important process during elongation. The wall properties are changed, but this will be an accompanying phenomenon.

In view of the results of these investigators and of the many various actions of growth substances on plants and plant cells, it does not seem likely that a direct action on the cell wall will be found. We may support FREY WYSSLING's opinion (1945, 1948, 1952) who maintained the importance of the protoplasm as an intermediary in the growth process.

3. THE INFLUENCE OF PRECEDING PLASMOLYSIS ON THE ACTION OF I.A.A.

It may be assumed that the action of I.A.A. will be affected by a preceding plasmolysis, as it is likely that at least part of the structural relation between cell wall and protoplasm will have been destroyed. To this end experiments were carried out in which sections were plasmolysed in 1 mol. mannitol. Microscopic examination showed that with this concentration of mannitol the parenchyma was completely plasmolysed. Occasionally even abnormal plasmolysis was observed. Plasmolysis could not be irrefutably established in the outer epiderm, owing to the great length of the cells, which makes it difficult to obtain intact cells. After 1 hour the mannitol was removed, the sections were rinsed thoroughly in distilled water and immersed in fresh distilled water, allowing deplasmolysis. The distilled water may contain I.A.A. so that the deplasmolysis took place in the presence of growth substance, or I.A.A. was added when the deplasmolysis had been completed. In table 18 and 19 the results are shown.

TABLE 18

The effect of preceding plasmolysis on elongation. The concentration of I.A.A. is 1 mg/l. The experimental numbers correspond to those of table 19.

Exp. no.	% Elongation				Duration of the exp. hours
	Plasmolysed sections		Not plasmolysed sections		
	I.A.A.	Contr.	I.A.A.	Contr.	
1	25,0	14,0			24
2	17,9	5,3	45,7	6,8	18
3 <i>d</i>	35,4	13,1			18
4 <i>a</i>	12,8	5,8	23,0		5
<i>b</i>	30,2	8,0	46,9		24
5	20,3	5,4	52,9	16,7	20
6	60,5	6,4	70,4	8,9	24
7	14,5	6,5	62,9	10,5	18

Preceding plasmolysis decreases the reaction of the elongation on I.A.A. (table 18), but a reaction still exists. Elongation can be resumed after plasmolysis. The extension is also increased by I.A.A., as appears from table 19. In some experiments this increase was appreciable, in others the effect was small or absent. Exp. 3 and 4 may indicate that a longer time between the plasmolysis and the addition of I.A.A.

favours the reaction of the sections, as the extension was maximum when the I.A.A. had been added 6 hours after plasmolysis. The analysis of this phenomenon will be difficult, for the variability increases as a result of plasmolysis. Therefore a further examination is in progress, employing the growth reaction proper.

TABLE 19
The effect of preceding plasmolysis on the elastic extension. The concentration of I.A.A. is 1 mg/l

Exp. no.	I	II	III	E			
				Plasmolysed		Not plasmolysed	
				I.A.A.	Contr.	I.A.A.	Contr.
1	0	0	24	7,6	7,0	11,7	7,9
2	0	6	18	6,3	5,3	12,0	6,4
3	0	0	24	9,4	7,2		
	0	2	22	10,1	6,9		
	0	4	20	10,0	7,5		
	0	6	18	11,6	8,3		
4	0	0	5	8,7	8,0		
	0	0	24	9,3	5,7		
	0	5	19	11,9			
5	4	0	20	8,2	5,8	11,7	7,9
6	5	0	24	13,2	7,3	13,5	6,9
7	0	6	18	6,3	6,0	13,2	7,1

I. Time between cutting and plasmolysing. II. Time between plasmolysis and the addition of I.A.A. III. Time of treatment with I.A.A.

It may be concluded that plasmolysis affects the action of I.A.A. This suggests an interaction between the protoplasm and the cell wall. The unexpected result, that sections still react on growth substances after plasmolysis and deplasmolysis may be explained by the preservation of a part of the structural relation, or by a partial recovery after deplasmolysis, or both possibilities may be realized. The first possibility is supported by the close association which is generally found between proteins and the primary wall.

4. THE INFLUENCE OF SUGARS ON THE ACTION OF I.A.A

It is a well known fact that sugars promote the action of growth substances (RIETSEMA, 1950). This can be seen in fig. 13 for example. Sugars are easily used in metabolism and therefore it seemed worthwhile to examine their effect on the elastic extension. A disadvantage of the use of these carbohydrates is that it takes 3 to 5 hours before a reaction of the growth rate on sugars can be found. The starving of sections before the addition of the sugars does not shorten this lag.

The results of a number of experiments in which glucose and sucrose were tested have been compiled in table 20. Sucrose and glucose appear to increase the effect of I.A.A. Without I.A.A. the effect of sugars on the elastic extension is not so evident. This corresponds to a slight reaction of the growth rate on sugars only. The

effect of sugars is very evident when the sections are immersed in distilled water for about 24 hours before the addition of the sugars. Apparently these starved sections are short of substrate.

TABLE 20

The effect of sugars on the elastic extension 1 mg/1 I.A.A. has been used. In column 2 the time between cutting and the addition of the sugars and I.A.A. has been given.

Exp.	Pretreat- ment hours	<i>E</i> %				Conc. sugar	Duration hours
		Contr.	Sugar	I.A.A.	I.A.A. sugar		
1	5	8,8	7,6	11,4	12,6	0,8 % glucose	20
2	5	6,6	8,1				24
3	20	4,9	7,1	9,6	15,9	"	20
4	24	6,1	7,2	11,9	12,8	"	7
5	24	4,3	11,1	8,2	13,8	"	28
6	24	5,1	5,9	9,7	14,4	"	24
7	5	6,3	6,8	14,7	16,7	1 % sucrose	20
8	6	8,1	7,8	13,0	14,4	"	19
9	5			10,2	15,4	"	24
10	6	7,9	8,2			"	23

In the above table the *E*-values are given and not the absolute value of the extension. The latter is with sugars 150 %–200 % higher than with I.A.A. alone, but part of this difference must be accounted for by the increased length attained after 24 hours. Fig. 13 (page 434), for example, shows that with I.A.A. plus sucrose the elongation amounts to more than 75 %, while I.A.A. alone gives 30 % elongation. Without any change in elastic properties the mere occurrence of such a difference of length will cause an increase in the elastic extension. As *E* is increased, however, the change in extension cannot be accounted for completely by the increase in length. The elastic properties must be changed.

This interaction between I.A.A. and carbohydrates indicates that the wall properties are changed through the intermediary of the protoplasm. In this relation the sugars may be used in the synthesis of new wall material.

5. THE INFLUENCE OF DINITROPHENOL ON THE ACTION OF I.A.A.

The preceding experiments suggest that the protoplasm has an active function in the change of the elastic extension by growth substances. The effect of sugars might be explained by assuming that the laying down of new material in the primary wall causes the increase of the elastic extension. In order to put this assumption to the test the effect of dinitrophenol (DNP) has been examined. BONNER (1949) and HACKETT and THIMANN (1950, 1952, 1953) have demonstrated that DNP inhibits growth and water uptake. It is shown in fig. 13 that DNP reduces the effect of both I.A.A. and I.A.A. plus sucrose on elongation. The first indication of growth inhibition is found 2–3 hours after the addition of DNP.

It had to be ascertained that DNP is not toxic to the sections. The

effect of different concentrations of DNP was therefore tested in combination with 1mg/l I.A.A. One of these experiments has been presented in fig. 14. In the experiment shown in this figure 3 mg/l DNP is already toxic, as the sections shrink after prolonged contact with DNP and cannot be plasmolysed any more. Moreover, these

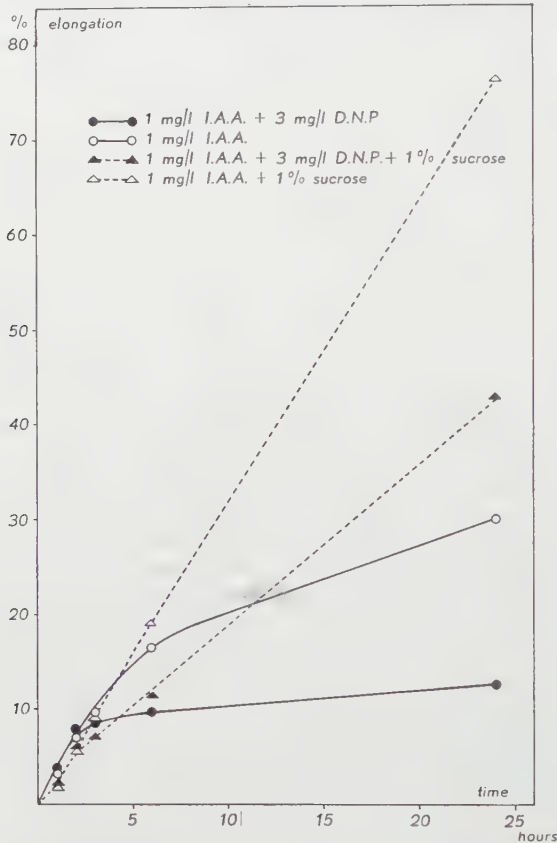


Fig. 13. The effect of DNP on elongation.

sections were not able to start growing again when the inhibitor had been removed. If measurements are made at the beginning of the experiments and after 18 or 24 hours only, as is commonly done, this phenomenon will escape observation, the more easily so, as the final length after the shrinkage is greater than the initial length in many cases. Conclusions based on such experiments run the risk of being incorrect. In this regard the results of BONNER (1949) must be reconsidered. Identical results have been obtained in the presence of I.A.A. plus sucrose. Lowering of the pH has the same effect as increasing the concentration of DNP (see table 22 exp. 4).

The sensitivity of the sections to DNP differs from day to day. In the experiment shown in fig. 14 the sections were very sensitive. Normally

3 mg/l does not show any damaging effect and often 4 mg/l can be used. A series of sections was always run for 18 to 24 hours to make sure that the DNP concentration used did not have an injurious effect.

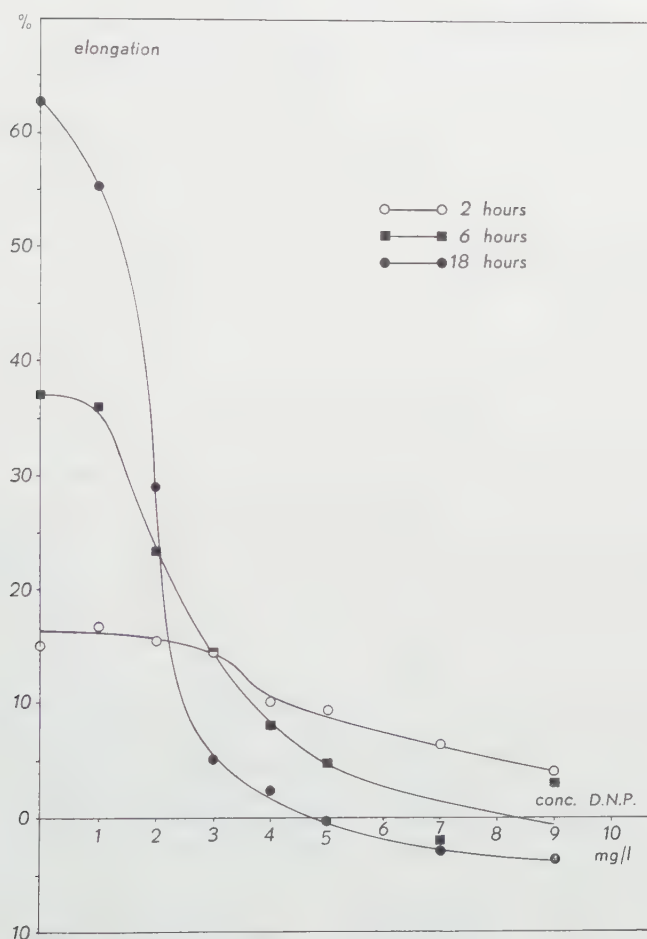


Fig. 14. The effect of different concentrations of DNP on elongation after 2, 6 and 18 hours of treatment. The sections received 1 mg/l I.A.A. together with DNP.

In a number of experiments the influence of DNP on the effect of I.A.A. and I.A.A. plus sucrose was examined. It appears from table 21 that growth is strongly inhibited and that E is decreased by DNP. The growth differences are very considerable, however, and this may complicate the interpretation. Therefore experiments were carried out in which the elastic extension and E were determined after 2 or 3 hours treatment. In this case the differences in growth are inconspicuous or do not exist at all. Differences in wall properties, if any, must therefore be brought about by the substances added.

In table 22 the results of these experiments have been collected, while exp. 6 is shown in fig. 15.

TABLE 21

The influence of DNP on the effect of I.A.A. and sucrose. Concentrations: 1 mg/l I.A.A., 1 % sucrose and 3 mg/l DNP

Composition of the medium	% elongation				E			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Dist. water.	15,3	5,6		7,1	8,1	8,3		6,6
DNP	8,9	7,6			3,2	7,1		
I.A.A.	62,5	27,5	29,9	42,0	13,0	10,1	10,2	13,3
I.A.A. + DNP. . . .	39,6	18,4	12,6	16,3	8,6	7,2	6,7	6,8
I.A.A. + suc.	105,0		76,3		14,4		15,4	
I.A.A. + suc. + DNP	57,7		43,0		12,9		13,2	
Duration of the exp. hours	19	18	24	18	19	18	24	18

TABLE 22

The effect of DNP on the elastic extension and on E in the presence of I.A.A. Concentrations used: 1 mg/l I.A.A. and 3 mg/l DNP. The values given are averages of 10 sections

Exp. no.	Elastic extension			E			Duration hours
	Contr.	I.A.A.	I.A.A.-DNP	Contr.	I.A.A.	I.A.A.-DNP	
* 1	3,9	6,4	4,7	9,3	13,8	10,7	2
2	4,0	5,4	4,5	9,6	12,3	10,2	2
			3,5 *			8,5 *	
† 3		4,5	3,1		10,6	7,5	2
4a		5,5	4,7		11,9	10,2	2
† b		5,4	3,9		11,8	8,8	2
5a	3,5	4,4	4,0 §	8,2	10,3	9,2 §	2
b	2,8	5,4	4,4 §	6,7	11,8	9,4 §	3
§ 6a	3,9	5,4	4,7	9,2	12,2	10,2	2
§ b	4,2	6,0	4,9	9,8	12,7	10,6	3

* 5 mg/l DNP

† In 1/100 mol KH_2PO_4 (pH 4,5).

§ Average value of 20 sections.

Considering these data it becomes apparent that DNP partly prevents the increase in the elastic extension, which is brought about by I.A.A. The effect of I.A.A. is decreased after 2 hours of treatment with DNP and even more after 3 hours. A low pH and a higher concentration accelerate the action of DNP (exp. 2, 3 and 4). A statistical analysis shows that the differences found are statistically significant or highly significant. For example, the chance that the very slight difference in exp. 5a originated by accident is about 7 in 1000.

The results of these experiments show that DNP intervenes with the action of I.A.A. on the cell wall. From the work of HOTCHKISS

c.s. (1944) it is known that DNP prevents the formation of high-energy phosphates and stops synthesis in the cell. This means that the change of the wall properties by I.A.A. is brought along via the synthetic activity of the cell.

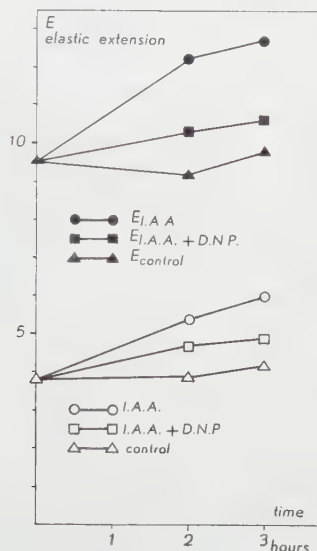


Fig. 15. The effect of 3 mg/l DNP on the action of 1 mg/l I.A.A.

6. DISCUSSION

The experiments described in this chapter suggest that the synthesis of wall substances is essential for the change in wall properties. The decreased effect of I.A.A. after preceding plasmolysis supports the assumption that I.A.A. acts on the cell wall through the intermediary of the protoplasm. The effect of carbohydrates points in the same direction, while the mechanism of the action is more specified by the fact that DNP affects the process. It may be tentatively concluded that growth substances stimulate directly or indirectly the synthetic activity of the protoplasm, which causes an increased synthesis of wall materials and protoplasmic constituents.

GENERAL DISCUSSION

As has been pointed out in the discussions in chapter II and III, an increase in the permeability to water or in the osmotic value of the cell contents cannot account for the higher growth rate of isolated coleoptile sections in the presence of I.A.A. As possible causes a change in the properties of the cell wall or a stimulation of an active process of water absorption are left. KRAMER and CURRIER (1950) stated that there is as yet no sufficient direct proof for the existence of an active water absorption. The new arguments put forward by THIMANN (1951)

did not provide binding evidence either (page 392). It is impossible, moreover, to estimate the part of an active "pumping" of water in the growth process and its connection with growth substances. Attention must, therefore, be focussed on the cell wall.

It has been stated (page 419) that the physical properties of the cell wall must change in order to maintain a high growth rate for some hours. An irreversible increase in area of the wall is the most economic, as then no energy is needed to keep the absorbed water in the cells (LEVITT, 1948). Either a change in the plasticity of the wall or a stimulated active growth in area of the primary wall might be effected by growth substances, which might increase the growth rate in this way. As MÜHLETHALER (1950) found that apposition of secondary layers with fiber texture occurs in still elongating cells, the first possibility is very unlikely. This is supported further by the invariate submicroscopic structure of the primary wall during elongation. Even the fast growing anthers of grasses (FREY-WYSSLING and SCHOCH-BODMER, 1938) and the seta of *Pellia* (OVERBECK, 1934. VAN ITERSOM, 1935) maintain the tube texture during elongation. Moreover, WIRTH (1946) and PRESTON and CLARK (1944) demonstrated that the amount of wall material increased during growth. The latter authors found that the amount of wall material per unit of length decreased, but this does not prove that elongation is a simple mechanical phenomenon.

The experiments reported in Chapter V of the present investigation give direct proof that the change in wall properties under influence of I.A.A. is connected with the metabolic activity of the cell. It appeared that DNP decreased the effect of I.A.A. on the elastic extension even when no growth differences could be found. This also indicates that no direct relation exists between the elastic extension and the growth rate. The difference in the time sequence of the extension and the growth rate after the addition of I.A.A. (page 426) points out the same. The change in elastic extension, however, indicates a change of the cell wall, a change which is not the consequence of elongation. The close oxygen dependency of the action of I.A.A. and the influence of preceding plasmolysis, carbohydrates and DNP on the effect of I.A.A. suggest that I.A.A. affects the cell wall through intermediary of the protoplasm.

For the mechanism of cell wall growth we may refer to MÜHLETHALER (1950) and FREY-WYSSLING (1950). MÜHLETHALER found that the individual cells of a coleoptile have inelastic centre parts and elastic poles, because secondary layers are absent at the tips of the cells only. The cells would elongate by bipolar tip growth by the interweaving of new cellulose fibrils. This conception is a modern variant of PFEFFER's "aktives Intussuszeptionswachstum" (1893). This hypothesis accounts for the high energy expenditure during elongation, as new fibrils must be synthesized.

The results of the present investigation may be explained by assuming that I.A.A. stimulates the biosynthesis of new fibrils and so the interweaving of these fibrils in the primary wall. MÜHLETHALER found

cells with long thin-walled poles in growing coleoptiles. In the presence of I.A.A. these poles might cover a larger area, for instance because the apposition of secondary layers temporarily lags behind. This will result in an increase in the elastic extension, as the elasticity is reduced by the secondary layers with fibre texture. The decrease in extension when the elongation slows down (fig. 10, page 423) might be explained by a relatively more pronounced apposition of secondary layers.

Comparable observations were made by EKDAHL (1953), who found that the extension of mature hair-cells of the root was about 1,5 %, while growing hair-cells were extended up to 11,2 %. In the root hairs the elastic extension decreased with the increase in length of the hairs. The growing apical wall was very extensible, but only within the very tip region less than 25 mikron long. EKDAHL also observed apposition of secondary layers immediately below the tip.

If the hypothesis outlined above is correct, the magnitude E (page 422) has little significance, as the completed parts of the wall attribute but a little to the elastic extension. In that case the extension is not directly proportional to the length of the cell. The effect of I.A.A. will then be much greater than appears from the E -values and will nearly account for the total absolute increase in the extension.

The plasticity of the newly formed parts of the wall may also be expected to be higher than that of the mature parts. This offers an explanation for the increase in plasticity after I.A.A. treatment found by HEYN (1931) and SÖDING (1934). The increase both in plasticity and in elasticity, however, will be secondary phenomena. These considerations are based on morphological studies of the cellulose skeletons of primary walls. What part the pectic substances play in the growth of the cell wall is still obscure. Some authors consider the pectic substances as the most essential part of the wall during growth (KERR, 1951. EKDAHL, 1953), but this assumption is still wholly unproven.

We do not know what metabolic processes are affected by growth substances and how the processes are linked together, no adequate techniques are available to study cell wall growth *in vivo* and we can only guess at the mechanism of the action of growth substances. It may be tentatively suggested that growth substances act on lipophilic phases or membranes within the protoplasm (ANKER, 1953). In the present investigation I.A.A. appeared to have a modifying effect on the ectoplasm. Within the protoplasm chondriosomes, for example, may be coacervates or may at least be separated from the remaining protoplasm by a complex-coacervate film. Such inner membranes may be affected by growth substances.

The final conclusion of the present paper is that elongation is an active growing of the cell wall by intussusception, which is affected by growth substances, presumably by a physico-chemical stimulation of one or more of the enzymatic processes leading to the biosynthesis of new cell wall materials. The synthesis of protoplasmic constituents during elongation (FREY-WYSSLING, 1945) may be stimulated in the same way. No need of an active (non-osmotic) water absorption

exists, for the osmotic value of the cell contents and the active growth of the cell wall will be able to maintain a suction force (page 000). Recently LEVITT (1953)¹ and BURSTRÖM (1953)² also did away with an active water absorption and with its possible function in the process of elongation.

SUMMARY

A study was made of the effect of indole-3-acetic acid (I.A.A.) on growth and water intake of isolated *Avena* coleoptile sections in distilled water. Both processes were greatly promoted by I.A.A. Attention was focussed on the mechanism of the action of I.A.A.

I.A.A. (1 mg/l) showed a tendency to decrease the permeability to heavy water, while a low pH increased this magnitude. The effect of I.A.A. and pH on growth and water intake could not be explained by their effect on the water permeability.

The sections always maintained a positive suction force, even after prolonged immersion in I.A.A. solutions or in distilled water. This suction force, which is found after prolonged immersion, originated from the active growth of the sections and was significantly increased after short times of treatment with I.A.A.

The osmotic value of the cell contents was not increased by I.A.A., but it decreased in proportion to the increase in length of the sections. I.A.A. did not stimulate the formation of osmotically active substances.

The elastic extension of the sections was increased by I.A.A. This indicated a change of the cell wall. It was demonstrated that the greatest increase in elastic extension occurred during the first two hours after the addition of I.A.A. No direct relation could be established between the elastic extension and the growth rate.

A further analysis showed that preceding plasmolysis decreased the effect of I.A.A. on the extension. Sucrose and glucose increased the effect of I.A.A., which increase might be partly due to a possible increase in the osmotic value by sugars. Dinitrophenol inhibited the action of I.A.A. on extension and on growth. These results indicated that I.A.A. changed the cell wall through intermediary of the active metabolism of the protoplasm.

It is suggested that elongation is an active growth in area of the cell wall by intussusception. I.A.A. is assumed to promote the biosynthesis of wall substances by a physico-chemical stimulation of one or more enzyme systems. This hypothesis accounts both for the high energy consumption and for the change in wall properties after the addition of growth substances to coleoptile sections.

¹ LEVITT, J. 1953. Further remarks on the thermodynamics of active (non-osmotic) water absorption. *Physiol. Plant.* 6 : 240.

² BURSTRÖM, H. 1953. Studies on growth and metabolism of roots. IX. Cell elongation and water absorption. *Physiol. Plant.* 6 : 262.

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REFERENCES

- ALBACH, W. 1930. Über die schädigende Wirkung der Plasmolyse und Deplasmolyse. *Protoplasma* 12 : 255.
- AMLONG, H. U. 1936. Der Einfluss des Wuchsstoffes auf die Wanddehnbarkeit der Vicia-Faba-Wurzel. *Ber. d. deutsch. Bot. Ges.* 54 : 271.
- AMLONG, H. U. 1939. Untersuchungen über Wirkung und Wanderung des Wuchsstoffes in der Wurzel. *Jb. f. wiss. Bot.* 88 : 421.
- ANKER, L. 1951. On the mechanism of auxin action II. The influence of indole-3-acetic acid on the respiration of starved *Avena* coleoptile sections. *Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam, Ser. C*, 54 : 525.
- ANKER, L. 1953. The effect of indole acetic acid and other growth promoting substances on the endogenous respiration of the *Avena* coleoptile. *Acta Bot. Neerl.* 2 : 22.
- AUDUS, L. J. 1952. The time factor in studies of growth inhibition in excised organ sections. *J. Exp. Bot.* 3 : 375.
- BARNES, T. C. 1934. The effect of heavy water of low concentration on *Euglena gracilis*. *Science* 79 : 370.
- BLANK, F. and H. E. DEUEL. 1943. Der Einfluss von Heteroauxin auf die Quellung von Membranzubstanzen. *Vierteljahrsschr. d. Naturf. Ges. Zürich* 88 : 161.
- BONNER, J. 1933. The action of the plant growth hormone. *J. Gen. Physiol.* 17 : 63.
- BONNER, J. 1934. Studies on the growth hormone of plants. V. The relation of cell elongation to cell wall formation. *Proc. Nat. Ac. Sci. U.S.A.* 20 : 393.
- BONNER, J. 1936. Zum Mechanismus der Zellstreckung auf Grund der Micellarlehre. *Jb. f. wiss. Bot.* 82 : 377.
- BONNER, J. 1949. Limiting factors and growth inhibitors in the growth of the *Avena* coleoptile. *Am. J. Bot.* 36 : 323.
- BONNER, J. and R. S. BANDURSKI. 1952. Studies of the physiology, pharmacology and biochemistry of the auxins. *Ann. Rev. Plant Physiol.* 3 : 59.
- BOOY, H. L. and H. VELDSTRA. 1949. Researches on plant growth regulators. XVI. The effect of plant growth substances on coacervates. *Biochim. Biophys. Acta* 3 : 260.
- BRAUNER, L. and M. BRAUNER. 1943. The relations between water intake and oxybiosis in living plant tissues. II. The tensility of the cell wall. *Rev. Fac. Sci. Istanbul, Sér. B*, 8 : 30.
- BRAUNER, L. and M. BRAUNER. 1943. Studies in the relations between water permeability and electric charge in membrane models and in living plant cells. *Rev. Fac. Sci. Istanbul, Sér. B*, 8 : 264.
- BRAUNER, L., M. BRAUNER and M. HASMAN. 1940. The relations between water intake and oxybiosis in living plant tissues I. *Rev. Fac. Sci. Istanbul, Sér. B*, 5 : 266.
- BRAUNER, L. and M. HASMAN. 1949. Über den Mechanismus der Wasseraufnahme von pflanzlichen Speichergewebe. *Bull. Fac. Méd. Istanbul* 12 : 57.
- BROOKS, S. C. 1935. The permeability of erythrocytes to Deuterium oxide (heavy water). *J. Cell. Comp. Physiol.* 7 : 163.
- BUFFEL, K. 1949. Onderzoekingen over groei en permeabiliteit bij het *Avena* coleoptiel. *Diss. Leuven* (unpubl.).
- BUFFEL, K. 1952. New techniques for comparative permeability studies on the oat coleoptile. *Meded. Kon. Vlaamse Acad. v. Wetensch. etc. (België)* 14, no. 7.
- BUNGENBERG DE JONG, H. G. 1947. In: KONINGSBERGER et al. *Leerboek der Algemene Plantkunde*, Vol. 2. Scheltema & Holkema, Amsterdam.

- BURSTRÖM, H. 1942. Die osmotische Verhältnisse während des Streckungswachstums der Wurzel. *Lantbr. Högskol. Ann.* 10 : 1.
- BURSTRÖM, H. 1942. The influence of heteroauxin on cell growth and root development. *Lantbr. Högsk. Ann.* 10 : 209.
- BURSTRÖM, H. 1951. Mechanisms of cell elongation. In: SKOOG. *Plant growth substances*. Madison Univ. Press, Wisconsin.
- CALDWELL, M. L., S. E. DOEBBELING and S. H. MANIAN. 1936. A study of the influence of heavy water upon the activity and upon the stability of pancreatic amylase. *J. Am. Chem. Soc.* 58 : 84.
- COGGESHALL, M. 1931. Influence of acetic, propionic, normal butyric and sulphuric acids and potassium acetate on elongation of primary roots of seedlings of white lupine. *Plant Physiol.* 6 : 389.
- COMMONER, B. C. and D. MAZIA. 1942. The mechanism of auxin action. *Plant Physiol.* 17 : 682.
- COMMONER, B. C. and D. MAZIA. 1944. The mechanism of auxin action: the effect of auxin and the C_4 -acids on salt and water absorption in *Avena coleoptile* and potato tuber tissue. *Am. J. Bot.* 31 : suppl. 8.
- COMMONER, B. C., S. FOGEL and W. H. MÜLLER. 1943. The mechanism of auxin action. The effect of auxin on water absorption by potato tuber tissue. *Am. J. Bot.* 30 : 23.
- DIEHL, J. M., C. J. GORTER, G. VAN ITERSOM and A. KLEINHOONTE. 1939. The influence of growth hormone on hypocotyls of *Helianthus* and the structure of their walls. *Rec. Trav. Bot. Néerl.* 36 : 709.
- EKDAHL, I. 1953. Studies on the growth and the osmotic conditions of root hairs. *Symb. Bot. Upsal.* 11 : 6.
- FRECKS, R. 1934. Das Wasserstoffisotop und das schwere Wasser. *Naturwiss.* 22 : 113.
- FREY-WYSSLING, A. 1945. Das Streckungswachstum der pflanzlichen Zellen. *Arch. Klaus. Stift etc. Festband ERNST* : 381.
- FREY-WYSSLING, A. 1948. Über die Dehnungsarbeit beim Streckungswachstum pflanzlicher Zellen. *Vierteljahrsschr. d. Naturf. Ges. Zürich* 93 : 24.
- FREY-WYSSLING, A. 1950. Physiology of cell wall growth. *Ann. Rev. Plant Physiol.* 1 : 169.
- FREY-WYSSLING, A. 1952. Deformation and flow in biological systems.
- FREY-WYSSLING, A. and H. SCHOCH-BODMER. 1938. Optische Analyse des Streckungswachstums von Gramineenfilamenten. *Planta* 28 : 257.
- GAST, A. 1942. Über den Einfluss der Dauer der Wachstumsstoffeinwirkung auf das Wurzelwachstum. *Ber. schweiz. Bot. Ges.* 52 : 441.
- GESSNER, F. 1934. Wachstum und Wanddehnbarkeit am *Helianthus-Hypokotyl*. *Jb. f. wiss. Bot.* 80 : 143.
- GESSNER, F. 1936. Phototropismus und Wanddehnbarkeit. *Jb. f. wiss. Bot.* 82 : 796.
- GUTTENBERG, H. von and A. BEYTHIEN. 1951. Über den Einfluss von Wirkstoffen auf die Wasserpermeabilität des Protoplasmas. *Planta* 40 : 36.
- GUTTENBERG, H. von and L. KRÖPELIN. 1947. Über den Einfluss des Heteroauxins auf das Laminargelenk von *Phaseolus coccineus*. *Planta* 35 : 257.
- GUTTENBERG, H. von and G. MEINL. 1952. Über den Einfluss von Wirkstoffen auf die Wasserpermeabilität des Protoplasmas. II. Über den Einfluss des pH-Wertes und der Temperatur auf die durch Heteroauxin bedingten Veränderungen der Wasserpermeabilität. *Planta* 40 : 431.
- HAAS, R. HORREÛS DE. 1929. On the connection between the geotropic curving and the elasticity of the cell wall. *Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam* 32 : 371.
- HACKETT, D. P. 1952. The osmotic change during auxin induced water uptake by potato tissue. *Plant Physiol.* 27 : 279.
- HACKETT, D. P. and K. V. THIMANN. 1950. The action of inhibitors on water uptake by potato tissue. *Plant Physiol.* 25 : 648.
- HACKETT, D. P. and K. V. THIMANN. 1952. The nature of the auxin-induced water uptake by potato tissue. *Am. J. Bot.* 39 : 553.
- HACKETT, D. P. and K. V. THIMANN. 1953. The nature of auxin-induced water uptake by potato tissue. II. The relation between respiration and water absorption. *Am. J. Bot.* 40 : 183.

- HASMAN, M. 1943. A study of the shape of the determinant curve in measurements of the suction potential in plant tissues. *Rev. Fac. Sci. Istanbul, Sér B*, 8 : 167.
- HEYN, A. N. J. 1931. Der Mechanismus der Zellstreckung. *Rec. Trav. Bot. Néerl.* 28 : 113.
- HEYN, A. N. J. 1940. The physiology of cell elongation. *Bot. Rev.* 6 : 515.
- HEYN, A. N. J. and J. VAN OVERBEEK. 1931. Weiteres Versuchsmaterial zur plastischen und elastischen Dehnbarkeit der Zellmembran. *Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam* 34 : 1190.
- HOTCHKISS, R. D. 1944. Gramicidin, Tyrocidine and Tyrothricin. *Adv. Enzymol.* 4 : 153.
- HSIANG, T. H. T. 1951. Physiological and biochemical changes accompanying pollination in orchid flowers. I. General observations and water relations. *Plant Physiol.* 26 : 441.
- HUBER, B. and K. HÖFLER. 1930. Die Wasserpermeabilität des Protoplasmas. *Jb. f. wiss. Bot.* 73 : 351.
- ITERTSON, G. VAN. 1935. The formation of the cell wall. *Proc. 6th Int. Bot. Congr. Amsterdam* 2 : 291.
- KELLY, S. 1947. The relation between respiration and water uptake in the oat coleoptile. *Am. J. Bot.* 34 : 521.
- KERR, T. 1951. Growth and structure of the primary wall. In: SKOOG, *Plant growth substances*.
- KÖGL, F. and A. J. HAAGEN SMIT. 1931. Über die Chemie des Wuchsstoffs. *Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam* 34 : 1411.
- KONINGSBERGER, V. J. 1942. De algemeene-biologische betekenis van vitaminen en hormonen. *Natuurk. Voordr. "Diligentia"*, Nieuwe reeks 20 : 13.
- KONINGSBERGER, V. J. 1947. Over de primaire werking van groeistoffen van het auxine type. *Meded. Kon. Vlaamse Akad. v. Wetensch. etc. (België)* 9 no. 13.
- KRAMER, P. J. and H. B. CURRIER. 1950. Water relations of plant-cells and tissues. *Ann. Rev. Plant Physiol.* 1 : 265.
- KROGH, A. and H. H. USSING. 1937. A note on the permeability of trout eggs to D_2O and H_2O . *J. Exp. Biol.* 14 : 35.
- LEVITT, J. 1948. The role of active water absorption in auxin-induced water uptake by aerated potato-discs. *Plant Physiol.* 23 : 505.
- LEWIS, G. N. 1934. The biology of heavy water. *Science* 79 : 151.
- LEWIS, G. N. and R. T. MACDONALD. 1933. Concentration of H^2 isotope. *J. Chem. Physics* 1 : 341.
- LÖVTROP, S. and A. PIÓN. 1951. Diffusion and active transport of water in the amoeba *Chaos Chaos*. *Compt. Rendu Lab. Carlsberg, Sér. chim.* 28 : 1.
- LUCKÉ, B. and E. N. HARVEY. 1934. The permeability of living cells to heavy water (Deuterium oxide). *J. Cell. Comp. Physiol.* 5 : 473.
- LUNDEGÅRDH, H. 1942. The growth of roots as influenced by pH and saltcontent of the medium. A contribution to the theory of growth. *Lantbr. Högsk. Ann.* 10 : 31.
- MEYER, B. S. 1945. A critical evaluation of the terminology of diffusion phenomena. *Plant Physiol.* 20 : 142.
- MEYER, S. L. 1934. Deuterium oxide and *Aspergillus*. *Science* 79 : 210.
- MÜHLETHALER, K. 1950. Elektronenmikroskopische Untersuchungen über den Feinbau und das Wachstum der Zellmembranen in Mais- und Haferkoleoptilen. *Ber. d. Schweiz. Bot. Ges.* 60 : 614.
- OVERBECK, F. 1934. Beiträge zur Kenntnis der Zellstreckung. Untersuchungen am Sporogonstiel von *Pellia epiphylla*. *Zschr. f. Bot.* 27 : 129.
- OVERBEEK, J. VAN. 1944. Auxin, water uptake and osmotic pressure in potato tissue. *Am. J. Bot.* 31 : 265.
- PARPART, A. K. 1935. The permeability of the mammalian erythrocytes to deuterium oxide (heavy water). *J. Cell. Comp. Physiol.* 7 : 153.
- PFEFFER, W. 1893. Druck und Arbeitsleistung durch wachsende Pflanzen. *Abh. math.-physik. Kl. III d. sächs. Ges. Wiss.* 20 : 404.
- PIÓN, A. and E. ZEUTHEN. 1951. The Cartesian diver balance in permeability studies. *Experientia* 7 : 455.
- POHL, R. 1948. Ein Beitrag zur Analyse des Streckungswachstums der Pflanzen. *Planta* 36 : 230.

- PRESTON, R. D. and C. S. CLARK. 1944. Wall structure and growth II. Wall deposition in *Avena coleoptiles*. Proc. Leeds Phil. and Litt. Soc., Scient. Sect. 4 : 201.
- REINDERS, D. E. 1938. The process of water intake by discs of potato tuber tissue. Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam 41 : 820.
- REINDERS, D. E. 1942. Intake of water by parenchymatic tissue. Rec. Trav. Bot. Néerl. 39 : 1.
- REINHARDT, M. O. 1899. Plasmolytische Studien zur Kenntnis des Wachstums der Zellmembran. Festchr. f. SCHWENDERER : 425.
- RESÜHR, B. 1935. Hydratations- und Permeabilitätsstudien an unbefruchteten *Fucus* Eiern. (*Fucus vesiculosus* L.). Protoplasma 24 : 531.
- RIETSEMA, J. 1949. A modified cylinder test of high sensitivity for growth substances. Proc. Kon. Ned. Akad. v. Wetensch. Amsterdam 52 : 1194.
- RIETSEMA, J. 1950. Action and penetration of growth substances. Diss. Utrecht.
- RUGE, U. 1937. Untersuchungen über den Einfluss des Hetero-Auxins auf das Streckungswachstum des Hypokotyls von *Helianthus annuus*. Ztschr. f. Bot. 31 : 1.
- RUGE, U. 1937. Untersuchungen über die Änderungen der osmotischen Zustandsgroszen und der Membraneigenschaften des Hypokotyls von *Helianthus annuus* beim normalem Streckungswachstum. Planta 27 : 352.
- RUGE, U. 1942. Zur Theorie der Mechanik der Zellstreckung und des Streckungswachstums. Planta 32 : 571.
- SAKAMURA, T. and H. KANAMORI. 1935. Über die Wirkung der Essigsäure, des Ammoniaks und ihre Salze auf das Protoplasma des Wurzelhaares. J. Fac. Sci. Hokkaido Imp. Univ. Sér. V, Bot. 4, no. 2.
- SANTEN, A. M. A. VAN. 1940. Groei, groeistof en pH. Diss. Utrecht.
- SEEMANN, F. 1949. Zur pH-Abhängigkeit der Wasserpermeabilität des Protoplasmas. Protoplasma 39 : 147.
- SÖDING, H. 1931. Wachstum und Wanddehnbarkeit bei der Haferkoleoptile. Jb. f. wiss. Bot. 74 : 127.
- SÖDING, H. 1932. Über das Streckungswachstum der Zellwand. Ber. d. Deutsch. Bot. Ges. 50 : 117.
- SÖDING, H. Über die Wachstumsmechanik der Haferkoleoptile. Jb. f. wiss. Bot. 79 : 231.
- SÖDING, H. 1952. Die Wuchsstofflehre.
- THIMANN, K. V. 1951. Studies on the physiology of cell enlargement. Growth 15 (suppl.) : 5.
- THIMANN, K. V. and J. BONNER. 1933. The mechanism of action of the growth substance of plants. Proc. Roy. Soc. London B 113 : 126.
- UREY, H. C., F. G. BRICKWEDDE and G. M. MURPHY. 1932. A hydrogen isotope of mass 2 and its concentration. Physic. Rev. 40 : 1.
- UREY, H. C., F. G. BRICKWEDDE and G. M. MURPHY. 1932. A hydrogen isotope of mass 2. Physic. Rev. 39 : 164.
- URSPRUNG, A. 1923. Zur Kenntnis der Saugkraft. VIII. Eine neue vereinfachte Methode zur Messung der Saugkraft. Ber. d. Deutsch. Bot. Ges. 41 : 338.
- URSPRUNG, A. and G. BLUM. 1924. Eine Methode zur Messung des Wand- und Turgordruckes, nebst Anwendungen. Jb. f. wiss. Bot. 63 : 1.
- VELDSTRA, H. 1944. Researches on plant growth substances V. Relation between chemical structure and physiological activity II. Contemplations on place and mechanism of the action of the growth substances. Enzymologia 11 : 137.
- VELDSTRA, H. and H. L. BOOY. Researches on plant growth regulators. XVII. Structure and activity. On the mechanism of action III. Biochim. Biophys. Acta 3 : 278.
- WARTIOVAARA, V. 1944. The permeability of *Tolypellopsis* cells for heavy water and methyl alcohol. Acta Bot. Fennica 34 : 1.
- WENT, F. W. 1928. Wuchsstoff und Wachstum. Rec. Trav. Bot. Néerl. 25 : 1.
- WEY, H. G. VAN DER. 1932. Der Mechanismus des Wuchsstofftransportes. Rec. Trav. Bot. Néerl. 29 : 381.
- WIRTH, P. 1946. Membranwachstum während der Zellstreckung. Ber. d. Schweiz. Bot. Ges. 56 : 175.
- ZEUTHEN, E. 1948. A Cartesian diver balance weighing reduced weights (R.W.) with an accuracy of $\pm 0,01 \gamma$. Compt. Rendu Lab. Carlsberg 26, 243.

THE INFLUENCE OF SALTS ON THE EXUDATION OF TOMATO PLANTS

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I. INTRODUCTION

In 1874 it had already been pointed out by SACHS that two types of exudation can be distinguished, i.e. the exudation which must be attributed to the occurrence of a local pressure in the stem, and the exudation caused by a process localized in the roots. The first phe-

nomenon is usually shown by trees. The second, although recorded also from trees, has been investigated mainly on herbaceous plants. We shall confine ourselves here to the exudation of decapitated plants, which is caused by the development of a root pressure. Many theories have been advanced on the causes of the development of root pressure. Some of them will be mentioned here. For a comprehensive review of older literature the readers are referred to HEYL's paper (1930); more recent literature has been summarized by KRAMER (1949) and ARNOLD (1952).

By many authors the water transport in exudation has been considered as a movement along gradients of increasing osmotic pressure. PRIESTLEY (1920) thought that cells adjacent to the vessels would have the highest concentration of osmotically active substances, so that water would be sucked in. As the cells between the endodermis and the vessels cannot expand, water would be pressed into the vessels. In this case it must be supposed, that the cells are more permeable to water on the inner than on the outer sides, a possibility which had already been mentioned by PFEFFER.

V. H. BLACKMAN (1921), however, pointed out, that it is not necessary to assume an osmotic gradient in the root tissue. According to his opinion the osmotic value of the cells should be of no importance, if only there is a difference between the osmotic value of the sap in the vessels and that of the medium. This view has been elaborated by SABININ (1925). He considered the action of the root as that of an osmometer. According to this author the rate of exudation should depend on the difference between the osmotic value of the medium and that of the sap in the ~~xylem~~ vessels, so that $b = k (P_x - P_e)$, b being the rate of exudation, P_x the osmotic value of the sap, P_e the osmotic value of the medium and k a constant. The root tissue must act as a semipermeable membrane. The values of P_x calculated from this formula agreed with the osmotic value of the sap.

RENNER (1915, 1929) stated like SABININ that changes in the osmotic value of the medium influenced the rate of exudation, in agreement with the osmometer theory. This was also confirmed by KÖHNLEIN (1930). EATON (1943) established in cottonplants a rectilinear relation between the rate of exudation and the difference between the osmotic value of the sap and that of the medium thus supporting SABININ's idea. KRAMER (1941) found that the exudation could be converted within one minute into absorption by enhancing the concentration of the outer solution. The reverse held true also. According to his opinion this can be explained only if the roots act as an osmometer.

Although these facts indicate the importance of an osmotic process for the transport of water, this cannot explain the whole process of exudation, as BLACKMAN has emphasized, since necessarily a certain concentration difference must somehow be maintained between the solution in the vessels and the external solution. According to PRIESTLEY the content of cells differentiating into xylem vessels would provide a supply of osmotically active material (mainly sugar). It has been proved however, that exudation sap generally contains little or

no sugar at all, but mainly salts. CRAFTS and BROYER (1938, c.f. also CRAFTS, CURRIER, and STOCKING 1949) supposed salts to diffuse through the symplast to the stele. Owing to the lower metabolic status (a result of the lower oxygen pressure) the cells in the stele cannot retain the same salt concentration as the cells in the outer layers of the root tissue, so that leakage of solutes to the vessels must take place causing a suction of water along the cell walls.

HOAGLAND and BROYER (1942; HOAGLAND, 1948) attributed root pressure to accumulation of salts and salt transport to the vessels. Since these processes are controlled by metabolism, the influence of oxygen and narcotics on the exudation would be explained. Nevertheless they thought it very probable that these phenomena could not be entirely explained in this way, especially on account of the influence of auxin on the exudation process, which had been found by SKOOG, BROYER and GROSSENBACHER (1938).

When investigating the exudation of tomato plants ARISZ, HELDER and VAN NIE (1951, c.f. VAN NIE, HELDER and ARISZ, 1950) started from the supposition that the exudation would consist of two processes: an active transport of salts to the xylem vessels and an osmotic suction of water by the xylem sap. If the osmotic value of the medium was altered, the rate of exudation showed a sudden change. A rectilinear relation could be demonstrated between the concentration of the medium and the sudden decrease of the rate of exudation caused by it. This provides an important support to SABININ's theory. Like this author they came to the conclusion that the rate of exudation was proportional to the difference between the osmotic value of the sap and that of the external solution: $b = k (O_b - O_m)$. The factor of proportionality, k , has to be considered as a measure of water conductivity of the root (b is the rate of exudation as in the formula of SABININ, and O_b and O_m are the osmotic value of the sap and that of the medium). In this case the rate of exudation would be controlled by two internal factors, k and the osmotic value of the sap. The latter depends on the secretion of salt into the vessels and on the transport of water, that is on k .

Like SABININ, RENNER and EATON, ARISZ, HELDER and VAN NIE found that if the osmotic value of the medium was enhanced, the sudden decrease of the rate of exudation was followed by a more gradual increase till a new equilibrium was attained. They pointed out that this could be explained by means of the theory mentioned above without assuming the permeation of substances from the medium. The fact is, that when the transport of water has been decreased by enhancement of the osmotic value of the medium, the sap must become more concentrated if the secretion of salts into the vessels continues at the same rate as before. This means an increase of the osmotic value of the sap and thus as the discrepancy with the osmotic value of the medium becomes greater, an increase of the rate of exudation also. Conversely a dilution of the sap, and a decrease of the rate of exudation are to be expected when the osmotic value of the medium is lowered. These changes of the osmotic value of the sap have actually been found.

The exudation phenomena, which were observed, could be explained for the greater part by means of the theory with which they started but there appeared to be one exception. The osmotic value of the sap was in most cases lower than would be expected. ARISZ, HELDER and VAN NIE did not consider this a reason to assume the occurrence of an active water transport as well as the active transport of salts. According to their opinion the transport of water and salts cannot be separated. In addition they mentioned the possibility that the osmotic value of the exudation sap is different from that of the sap in the vessels of the roots.

In the papers which have been discussed above, the exudation was considered as the result of a difference of osmotic value between the sap in the vessels and the medium. Now we must mention the view of some authors who hold another opinion.

SPEIDEL (1939) considered the salt transport of no importance. His idea concurs more or less with that of PRIESTLEY: the cells surrounding the vessels should have the highest osmotic value and attract water. By enzymatic processes, osmotically active substances are broken down continually and in consequence of this the osmotic value decreases, so that water which had been bound osmotically is pressed into the vessels.

VAN OVERBEEK (1942) distinguished an osmotic as well as an active water transport. He found, that the concentration of the external solution that would fully inhibit the exudation of tomato plants, was always much higher than the osmotic value of the exudation sap. This would indicate the presence of an active component. Only the latter was sensitive to potassiumcyanide. ARISZ, HELDER and VAN NIE however offered some criticism on VAN OVERBEEK's method of determining the root pressure. BROYER (1951) following VAN OVERBEEK's method did not succeed in obtaining wholly satisfactory results.

ARNOLD (1952) concluded from facts in the literature on the uptake and secretion of water and salts that the transport of water should take place entirely independently from the transport of salts. The protoplasm of the cells would, according to his opinion, take up water both osmotically and electroosmotically. The intensive transport of water through the endodermis, however, was considered by him to be an active process, which could also take place against a concentration gradient.

The theory of LUNDEGÅRDH (1945, 1949, 1950) holds more or less a position of its own. This author considered the transport of salts of importance for the exudation of wheat plants, but rejected his former view that the transport of water would be caused by the difference between the osmotic value of the sap and that of the external solution (1940, 1943). According to his opinion anion respiration, pumping salts into the cells, should be the driving force of the exudation process: "Anion respiration and a comparatively high exchange permeability together build up the steady state of salts, viz. the concentration level of salts in the root tissue, the former representing the up-hill reaction, the latter the down-hill reaction". The exudation

of salts from the cells is to be considered as a passive process. When salt is given off from the cells, there is a change in their osmotic value. The turgor is decreased and besides salt, water is expressed. This salt solution is exuded by the vascular epithelium, the cells adjacent to the xylem vessels.

The movement of water is impeded by an increase of the osmotic value of the medium. This causes the exudation of a smaller amount of the salt solution, but the concentration of the solution is not changed. According to LUNDEGÅRDH's view, besides this solution, water without salt is given off to the vessels. This is water, which has been released when osmotically active substances are converted into inactive substances in synthetic processes. This so-called extra water secretion is inhibited by sodium fluoride, which proves that the process is connected with glycolysis. In the third place water together with carbon-dioxide, produced in respiration, can be transferred to the vessels. Afterwards the carbondioxide can escape from the vessels.

The experiments, which are described below, form a continuation of the investigation of ARISZ, HELDER and VAN NIE.

The osmometer theory has been used as a working hypothesis; that is we have supposed that the exudation process is based on a transport of salts to the vessels causing a concentration difference between the xylem sap and the medium, which results in an osmotic transport of water.

Because of the possibility of a close relation between water and salt transport (ARISZ, HELDER and VAN NIE; LUNDEGÅRDH) it seemed logical to try in the first place to obtain a better understanding of the process of salt secretion. To this end the influence of several factors on the process has been investigated. The effect of changes in the salt concentration of the outer solution proved to be very important. This can be investigated in various ways. From the observed changes in the rate of exudation, conclusions may be drawn concerning the changes in the salt secretion. On the other hand the salt secretion has been investigated more directly by determining the composition and the salt concentration of the exudation sap. A second point for investigation was constituted by the question to what extent salt secretion and exudation would be influenced in circumstances, where active processes should be stimulated (sugar supply) or inhibited (presence of inhibitors in the medium). Our working hypothesis will be discussed in connection with the facts obtained in the experiments.

The investigation was made at the Botanical Laboratory at Groningen. I wish to express my thanks to Professor Dr W. H. Arisz for his valuable advice and the interest taken in my work. To Mr G. C. Mees, Nottingham, I am greatly indebted for his correction of the English text of this paper.

II. MATERIAL AND METHODS

The experiments were performed with tomato plants and *Sanchezia nobilis*.

Tomato plants about six weeks old were rinsed with water to remove soil particles from the roots. By means of a cork, which had been pierced and split afterwards, the plants were fixed into a hole (diameter 2–3 cm) that had been bored into a wooden disk. The disks were placed on pots containing approximately one litre of Hoagland solution. This nutrient solution contains 0.0025 M. KNO_3 , 0.0025 M. $\text{Ca}(\text{NO}_3)_2$, 0.001 M. MgSO_4 and 0.0005 M. KH_2PO_4 in tap water (HOAGLAND and BROYER, 1936). To one litre of solution 1 ml A-Z solution was added. The pH of the solution was adjusted to about 6.2 by adding diluted sulfuric acid. When necessary, that is about every three weeks, iron was given as iron tartrate, 1 ml of a saturated solution to one litre Hoagland solution. In that case a nutrient solution without phosphate was used.

The nutrient solution was replaced every week. If necessary the pots were replenished with tap water in the time between.

In the summer of 1951 the water cultures were placed in a green-house. After 6–8 weeks the plants had developed enough roots to be used in experiments. During the last two weeks the solutions were aerated.

In 1952 the use was obtained of a small green-house, that could be illuminated artificially (VAN DER VEEN, 1950). Thus it was possible to cultivate the plants under more constant conditions, at least in regard to the lighting. Now, too, plants could be cultivated in winter.

For practical reasons the green-house was illuminated from 9 in the evening till 11 in the morning. The temperature rose to 24° C during the light period and fell to 10°–18° C in the dark, depending on the outside temperature.

Owing to lack of room the plants could not be cultivated all the time in this green-house. Usually they were placed in it, when they had been put on a nutrient solution; in winter, however, younger plants still growing in soil were also placed in the green-house. The plants developed well under these conditions, better usually than the plants which were grown in a normal green-house. They could be used after having been on a nutrient solution for 5–6 weeks. The plants showed a stronger exudation and exuded for a longer time, so that often the same plant could be used during two or even three successive days.

Cuttings of *Sanchezia nobilis* were put on pots filled with tap water after having been fixed in the hole of a wooden disk in the same way as tomato plants. It might take several weeks before roots began to develop. After that the plants were put on a Hoagland solution. In an ordinary green-house the root system had developed sufficiently after 2–4 months depending on the season. In the artificially lighted green-house this took a good two months.

1–3 ml sap was exuded by these plants in an hour. The same plant continued to exude sap for several days, sometimes a week. The plants were cut off some cm above the place where the first roots grow out of the stem. Usually they were decapitated the evening before the day on which the experiment was to take place. Still fixed in the wooden

disk they were placed on a large funnel, whose outlet had been closed by means of a rubber tube with pinchcock. Into the wooden disk two smaller holes had been bored beside the large one. The funnel could be filled through one of them, while the other served for the aeration.

The solution in the funnel could be replaced within one minute. During the night before the experiment the plants were usually put on a Hoagland solution. Next morning this solution was renewed. Once again a piece of the stem was cut off and then a capillary tube was placed on the stump and fixed by means of a rubber tube. The capillary tube consisted of a short vertical and a longer horizontal part, connected by a three-way glass tap. The horizontal part of the tube could easily be emptied by blowing through the tap.

The movement of the meniscus of the sap in the horizontal part of the tube was read off every 30 seconds, with interruptions of a few minutes, if the tube had to be emptied. The tube was calibrated in mm. The reading was estimated to 0.1 mm. One mm corresponded with approximately 1 mm³ of liquid.

In this way it was possible to follow in detail the changes of the rate of exudation. It appeared from the investigation of ARISZ, HELDER and VAN NIE that the precise course of the change in the rate of exudation could often be of great importance.

Besides the rate of exudation the osmotic value of the sap was determined. Right over the stump a hole was pricked into the rubber tube by which the capillary had been fixed on the stump. A sample of the outflowing sap could be taken through this hole by means of a narrow glass capillary (about 7 cm long, diameter ± 0.4 mm). If the rubber was sufficiently elastic, the hole closed again on its own account, otherwise it was filled up with some vaseline. Leakage seldom occurred and was perceptible in a short time.

The samples of the sap were preserved in the capillary tubes whose ends had been closed with vaseline, till the osmotic value could be determined. This was done the same day as the samples had been taken.

As a rule the osmotic value of the sap was not determined before a plant had been on a solution for half an hour or more. It appeared that when the circumstances had been altered, it took 20 minutes or more before the osmotic value of the sap was constant again. When the rate of exudation was less, it took more time, before this moment was reached (VAN ANDEL 1952).

The osmotic value of the sap was determined by means of a thermoelectric osmometer according to BALDES and JOHNSON. The principle of this method is as follows: if two drops of solutions of different osmotic values are placed in a container saturated with water vapour, the temperature of the drops is changed to a different extent. The resulting temperature difference between the drops, which is dependent on the difference between their osmotic values, is measured by means of a thermocouple. The apparatus that is used for this purpose, has been described in more detail elsewhere (BALDES and JOHNSON 1939; VAN ANDEL 1952). A small amount of liquid — ± 0.01 ml — is

required for one determination, so that determinations can be made every now and then, whereas one would have to use up more than 1 ml sap in other methods of determining the osmotic value. A second advantage of this method is, that the determinations take relatively little time. One complete determination takes about one hour, as the drops have to be in equilibrium with their surroundings, but when several thermocouples are available, 5-6 determinations can be made at the same time.

Every thermocouple must be calibrated with known solutions; in this case solutions of boric acid were used. The osmotic value of the sap was always expressed as the concentration of the solution of boric acid, with which it is isotonic. The osmotic values of the external solutions are placed between brackets in this paper. If for instance a 0.005 M. KNO_3 (0.011 M.) solution is referred to hereafter, a solution is meant that contains 0.005 M. KNO_3 and is isotonic with a 0.011 M. solution of boric acid. The determinations proved to be sufficiently accurate. If the osmotic value of the solutions was less than that of a 0.05 M. boric acid solution the error was approximately 0.001-0.002 M boric acid. When the sap or the solutions were more concentrated a larger error was found.

In addition to the rate of exudation and the osmotic value of the sap the water conductivity of the roots was sometimes determined in the way, indicated by ARISZ, HELDER and VAN NIE. According to these authors the factor k in the formula $b = k (O_b - O_m)$ should be a measure of its magnitude. When in certain conditions the rate of exudation is b_1 , the osmotic value of the sap O_b , and that of the medium O_{m1} , $b_1 = k (O_b - O_{m1})$. When the osmotic value of the medium is enhanced to O_{m2} , this causes a decrease of b_1 to b_2 . Now $b_2 = k (O_b - O_{m2})$. This applies to the moment immediately after the change of the medium, when O_b has not yet changed. We can find k now by subtracting the equations: $b_1 - b_2 = k (O_{m2} - O_{m1})$ or $k = (O_{m2} - O_{m1}) / (b_1 - b_2)$. We know $b_1 - b_2$, the sudden decrease of the rate of exudation. $O_{m2} - O_{m1}$ can be calculated. So k can be determined if a solution of a known osmotic value is replaced by another solution, whose osmotic value is also known.

When sap was to be collected for determinations of the salt concentration, a capillary tube, that had been bent twice at right angles was placed on the stump of the plant. Under the end of the tube a 25 ml container was placed to receive the sap.

The amount of sap, exuded in a certain space of time, was determined by weighing. For determining the concentration of the sap the specific gravity was supposed to be one. The specific gravity proved to be somewhat greater, so the concentrations that have been calculated in this way, are a little too low.

When the amount of sap had been determined the container was topped up with glass distilled water to a volume of 25 ml. The nitrate and phosphate concentrations of the diluted sap were determined colorimetrically: nitrate by the phenoldisulphonic acid method, phosphate according to the molybdenum blue method. Sometimes the

chloride was determined by microtitration according to Volhardt.

The pH of the sap and of the solutions was determined by means of a Beckman pH meter.

In some cases the conductivity of the sap was determined by means of a Philoscoop. The concentrations, determined in this way, showed a good agreement with the concentrations calculated from determinations of the osmotic value of the solutions by means of the thermo-electric osmometer.

In most experiments a Hoagland solution was used, that had been made with distilled water. The solution did not contain iron or A-Z solution. The pH amounted to 5.2. Solutions, that did not contain phosphate, had a somewhat higher pH (± 6). In the figures and tables a Hoagland solution is referred to as H, a solution containing no salts but mannitol only as M.

During the experiments the solutions were continually aerated. The experiments were performed in a cellar. The temperature was not quite constant, but showed a rise throughout the day. The changes, however, were very slight. The determinations of the osmotic value were done in the same room, but then a water bath was used, whose temperature could be kept constant.

III. DESCRIPTION AND DISCUSSION OF THE EXPERIMENTS

§ 1: INFLUENCE OF CHANGES IN THE OSMOTIC VALUE AND THE SALT CONCENTRATION OF THE MEDIUM ON THE EXUDATION

Experiment 1 (fig. 1). A plant was put alternately on a Hoagland solution and on an isotonic solution of mannitol. When the Hoagland solution was changed for one of mannitol, the rate of exudation, which had previously been constant, immediately decreased. The rate of this decrease declined till after about 20 minutes the rate of exudation was again constant. At this time the value was approximately two thirds the original value. The mannitol solution was next replaced by the Hoagland solution: now the rate of exudation began immediately to increase till it was at last just as great as before in such conditions.

It appears from table 1 that the osmotic value of the sap, O_b , was a little lower when the outer solution did not contain any salt, but only mannitol. The product of osmotic value and rate of exudation, which is a measure of the amount of salt given off to the vessels every 30 seconds (S , expressed always as $\text{mm}^3 \times M/30 \text{ sec.}$) showed a decrease. The results of several similar experiments have been summarized in table 1.

Osmotic value and salt secretion into the vessels were determined when the plants had been on a certain solution for about half an hour. As only 0.01 ml of sap was required for a determination by means of the thermo-electric osmometer, the osmotic value of the sap could have been determined sooner, in fact a few minutes after the medium

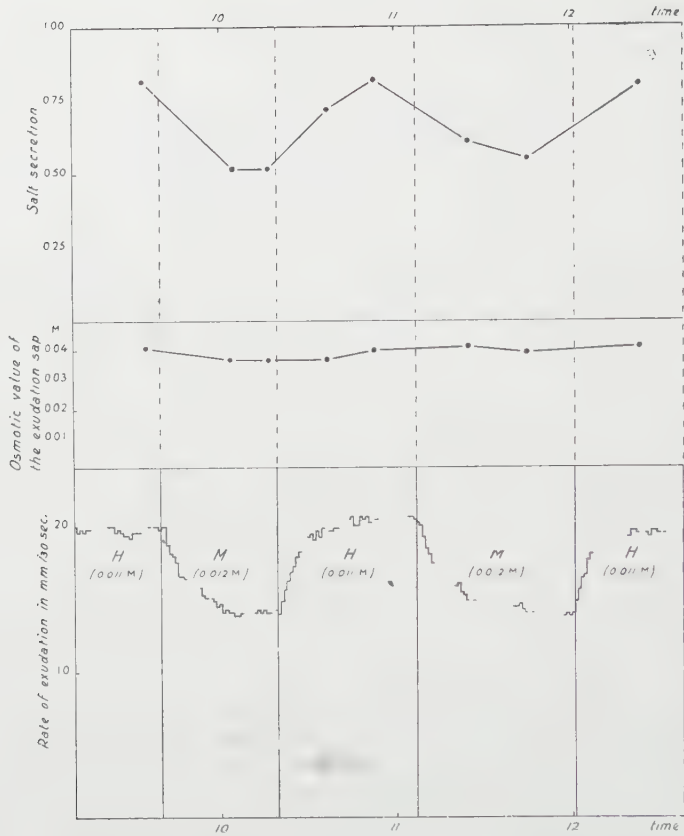


Fig. 1. Changes in the exudation caused by replacing a salt solution by an isotonic solution lacking salts, and vice versa. The rate of exudation is represented in the lower part of the figure; the osmotic value of the sap in the middle part and the salt secretion in the upper part.

TABLE 1

Changes of the salt secretion into the vessels as a result from a change of the salt concentration of the medium. The outer solutions were a Hoagland solution (H) and an isotonic solution of mannitol (M).

Experiment	Medium	H	M	H
1	O _b	0.041	0.037	0.040
	S	0.82	0.57	0.82
	O _b	0.040	0.039	0.041
	S	0.82	0.55	0.82
1a.	O _b	0.048	0.041	0.048
	S	0.96	0.54	0.96
1b.	O _b	0.042	0.038	0.041
	S	0.81	0.54	0.96
1c.	O _b	0.044	0.038	0.047
	S	0.66	0.33	0.69
1d.	O _b	0.044	0.035	0.042
	S	1.19	0.60	1.08

O is expressed as M. boric acid; S as mm³ × M boric acid/30 sec.

had been changed. It appeared, however, that it took some time before the exuding sap showed the changes, which had taken place in the sap in the vessels of the roots as a result of changing the medium. About 300–400 mm³ of sap must flow out before sap of the new concentration reaches the cut surface (VAN ANDEL, 1952).

It appears that if the plants were on a Hoagland solution the amount of salt given off to the vessels was approximately one and a half or two times as large as that which was given off when the outer solution did not contain any salt. This means that the salt secretion diminished considerably if no salt could be taken up, but did not cease altogether. Even when a plant had stayed on a mannitol solution for more than an hour exudation and salt secretion were still very much as they were after the state of equilibrium had been attained.

The rate of exudation was reduced by smaller decreases in the salt concentration of the medium as well as by removing all the salts.

In experiment 2 (fig. 2) the exudation was investigated on solutions containing salts in the same proportion as the Hoagland solution but in different concentrations (0; 0.1; 0.2; 0.5 and 1 H). The solutions had been made isotonic by adding mannitol. Every decrease in the salt concentration of the medium caused a decrease in the rate of exudation. The behaviour was always the same: the rate of exudation decreased rapidly, afterwards more slowly, and became constant after about 15 minutes. The rate of exudation at the state of equilibrium became smaller as the outer solution contained less salt.

The reduction of the rate of exudation was usually accompanied by a decrease of the osmotic value of the sap and therefore by a decrease in the salt secretion too.

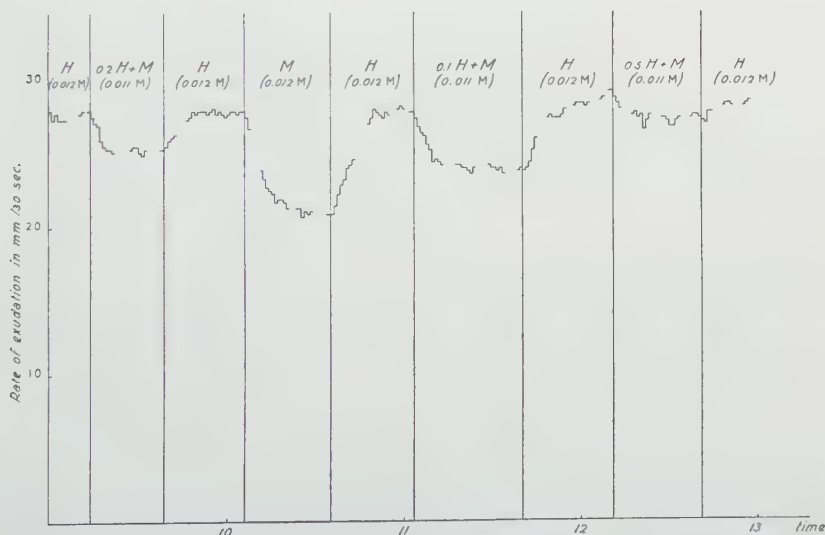


Fig. 2. Relation between the salt concentration of the medium and the rate of exudation.

Changes of the salt concentration of the medium apparently always cause a change of the rate of exudation.

It has been mentioned in the introduction, that changes of the osmotic value of the medium can also cause a change in the rate of exudation. In this case however the changes take another course: enhancing the osmotic value of the medium causes a sudden decrease in the rate of exudation followed by a more gradual increase to a constant level, which is lower than the previous equilibrium value. Conversely the rate of exudation can be suddenly increased by a lowering of the osmotic value of the outer solution, after which a gradual decrease follows (ARISZ, HELDER and VAN NIE). What will happen now, if salt concentration and osmotic value of the medium are changed at the same time?

Experiment 3 (fig. 3). In the left part of the figure we can see the effect of an increase in the osmotic value of the medium, which is just as has been described above. The salt concentration was not

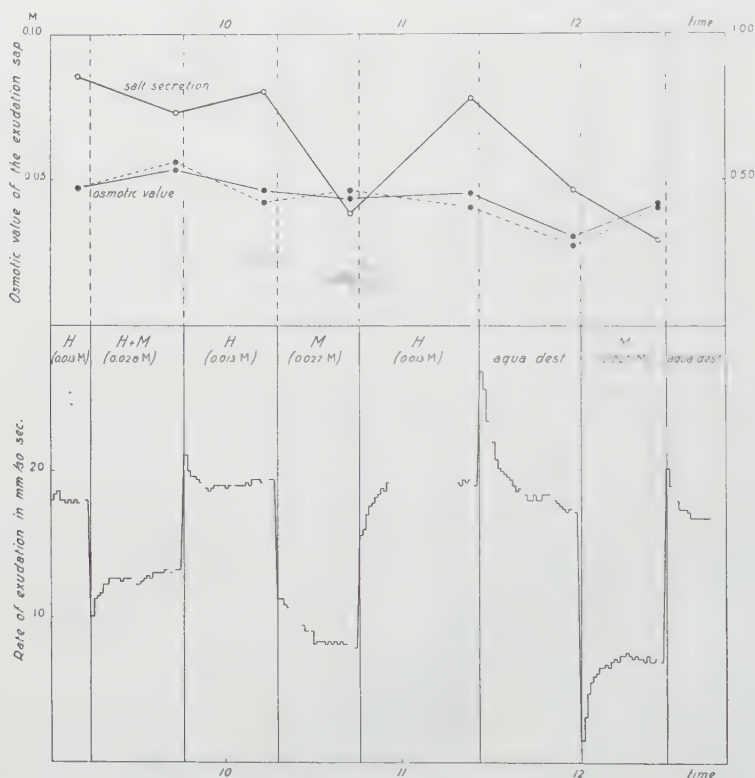


Fig. 3. Influence of changing the osmotic value of the medium on the rate of exudation and salt secretion, compared with the effect of a simultaneous change in the osmotic value and the salt concentration of the outer solution. The rate of exudation is represented in the lower part of the figure; the osmotic value of the sap (●-----● experimental data; ●-----● data calculated from the formula) and the salt secretion (○-----○) in the upper part.

altered in this case. When next the osmotic value of the outer solution was again enhanced but now the salt concentration lowered at the same time by replacing a Hoagland solution by a hypertonic solution of mannitol, the rate of exudation showed a sudden decrease as before. A further more gradual decrease followed the sudden fall. At last a constant level was attained.

Lowering the osmotic value of the medium and addition of salts caused a sudden and then a more gradual increase in the rate of exudation, till the same value had been attained as before on a Hoagland solution.

After this the plant was put on distilled water, which meant a decrease of the osmotic value and of the salt concentration of the medium (sixth period of experiment 3). The sudden increase of the rate of exudation was followed now by a gradual decrease. When next the water was replaced by the mannitol solution the result was the same as when mannitol was added to a Hoagland solution (second period): the rate of exudation was suddenly diminished, but afterwards it increased gradually.

In the first and the last part of the experiment only the osmotic value of the medium was altered; the changes of the rate of exudation took an analogous course in both cases. When, however, the change of the osmotic value was accompanied by a change of the salt concentration as in the third and fourth period, the rate of exudation changed in another way. The equilibrium which was finally attained on a certain solution — a mannitol solution for instance — was the same whether it had been attained by changes of the osmotic value alone, or of the osmotic value and the salt concentration together (fig. 3 fourth and seventh period).

The same phenomenon as has been described just now, is demonstrated in the first part of experiment 4 (fig. 4). In the second part (sixth period) a more concentrated solution of mannitol (0.059 M.) has been used. It appeared that the rate of exudation now changed in a way analogous to that when a solution of mannitol and salts was used (second and eighth period): the rate of exudation decreased first and increased gradually afterwards. The mannitol solution was next replaced by a Hoagland solution. This now caused a sudden increase and gradual decrease.

Another course of the rate of exudation is shown in experiment 5 (fig. 5). In this case a Hoagland solution was replaced alternately by mannitol solutions of different concentrations. The lowest concentration (0.018 M.) caused a sudden decrease followed by a more gradual decrease of the rate of exudation, a phenomenon already known from fig. 3 and 4. The most concentrated solution (0.055 M.) caused the exudation to decrease at first, but afterwards there was a gradual increase just as in fig. 4 (sixth period). When, however, a solution was added whose osmotic value was 0.025 M., the rate of exudation fell suddenly but then stayed at the same level; only after 10 minutes did it decrease somewhat further. On the Hoagland solution (third period) the sudden increase in the rate of exudation

was followed by a further very slight increase. In these experiments the osmotic value of the sap was determined in addition to the rate of exudation. This was done just before the external solution was changed. The osmotic values of the sap in experiments 3 and 4 are summarized in table 2, together with the results of some similar experiments.

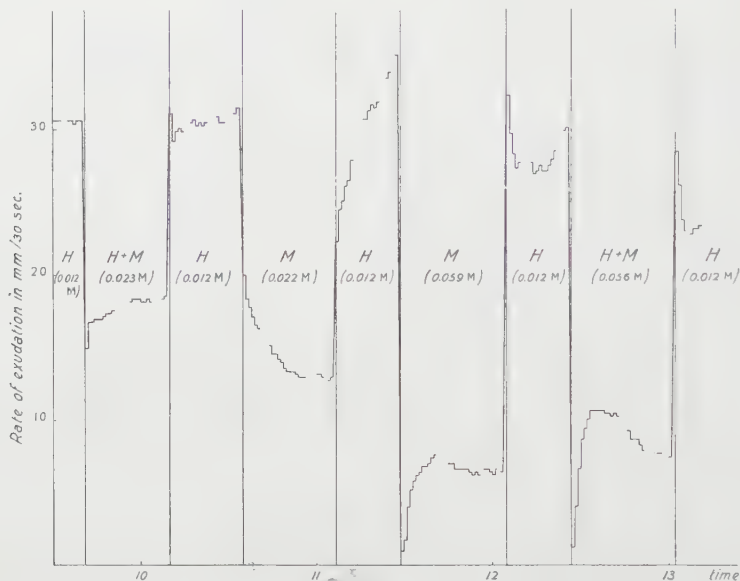


Fig. 4. Comparison between the effects of various changes in the osmotic value of the medium on the rate of exudation in the presence and absence of salts.

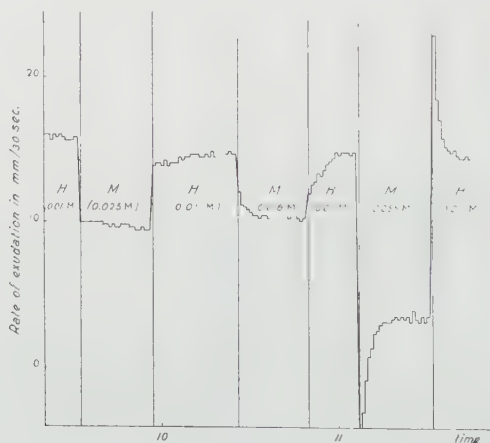


Fig. 5. Relation between the course of the exudation and the change in the osmotic value of the medium. Changes of the osmotic value of the medium are accompanied by changes in the salt concentration, so that water and salt transport are affected simultaneously.

The osmotic value of the sap increased, when the osmotic value of the medium was enhanced, the salt concentration not being changed. Thus the osmotic value of the sap showed a rise from 0.043 M. to 0.053 M. when the osmotic value of the medium was increased from 0.012 M. to 0.023 M. (experiment 4). This agrees with the results of ARISZ, HELDER and VAN NIE. When in the same experiment the osmotic value of the medium was enhanced to 0.022 M. and the salt was removed from the outer solution, the osmotic value of the sap increased, but to a smaller extent, that is from 0.042 M. to 0.049 M. From table 2 it appears that in similar cases the osmotic value of the sap can also stay unaltered (experiment 4a, 4c) or decrease somewhat.

When a concentrated solution of mannitol was used for the outer solution the osmotic value of the sap always increased, but not to the same extent as when a solution of salt and mannitol of the same osmotic value replaced the Hoagland solution (experiment 4, sixth and eighth period).

In all cases less salt was given off to the vessels, when the outer solution did not contain salt. If we want to compare the values of the salt secretion in different circumstances we must reckon with the fact, that an enhancement of the osmotic value of the medium alone can reduce the salt secretion. Only when the outer solutions differ merely in salt concentration is it possible to get a correct idea about the influence of the salt concentration of the medium on the salt secretion. The influence of the osmotic value of the medium on the salt secretion will be discussed in section 5.

In the experiment described above tomato plants were used. The same results were obtained with *Sanchezia nobilis*, as can be seen from table 2 (experiment 4c). Here also less salt was given off to the vessels when a Hoagland solution was replaced by a hypertonic solution of mannitol. The sudden fall of the rate of exudation was followed by a further more gradual decrease, in the same way that has been demonstrated in fig. 3 and 4 (fourth period).

It may be observed that these phenomena are not due to a specific action of mannitol, since sucrose has just the same effect (experiment 14).

Thus the rate of exudation proves to be influenced by changes in the salt concentration of the medium. The way in which the rate of exudation is changed depends on the extent of the simultaneous change in the osmotic value of the medium.

How are we to explain the phenomena set forth above?

According to the theory of ARISZ, HELDER and VAN NIE there are two possible causes for a change of the rate of exudation. In SABININ's formula $b = k (O_b - O_m)$ either k can change or $O_b - O_m$, so that either the conductivity of the roots for water or the difference between the osmotic value of the sap in the vessels and that of the medium must have altered.

Let us first consider the former possibility. When the outer solution is changed k can be determined by dividing the sudden change in the

TABLE 2
Changes in the osmotic value of the sap and the salt secretion caused by simultaneous changes
of the salt concentration and the osmotic value of the medium

Exper.	Medium	H	H + M(0.023 M)	H	M(0.022M)	H	M(0.059M)	H	H + M(0.056 M)	H	M(0.016M)
4	O_b	0.043	0.053	0.043	0.049	0.042	0.056	0.042	0.060		
	$O_b(\text{theor})$	0.046	0.054	0.043	0.037	0.050	0.058	0.058	0.074		
	S	1.32	0.99	1.37	0.64	1.43	0.36	1.26	0.44		
4a	Medium	H	H + M(0.056 M)	H	M(0.049M)	H	M(0.031M)	H	M(0.073M)	H	M(0.016M)
	O_b	0.049	0.067	0.049	0.053	0.047	0.048	0.046	0.061	0.043	0.035
	$O_b(\text{theor})$	0.059	0.076	0.055	0.067	0.052	0.062	0.057	0.080	0.040	—
4b	Medium	H	H + M(0.027 M)	H	M(0.020M)	H	distill. water	1.60	0.51	1.40	0.85
	O_b	0.026	0.041	0.027	0.033	0.035	0.022	0.046	0.030		
	$O_b(\text{theor})$	0.038	0.041	0.035	0.030	0.043	0.027	0.057	0.035		
4c	Medium	H	H + M(0.029 M)	H	M(0.031M)	H	0.51				
	O_b	0.034	0.043	0.036	0.036	0.036					
	$O_b(\text{theor})$	0.055	0.072	0.059	0.051	0.062					
4d	Medium	H	H + M(0.037 M)	H	M(0.037M)	H					
	O_b	0.050	0.064	0.048	0.059	0.049					
	$O_b(\text{theor})$	0.070	0.083	0.071	0.067	0.075					
	S	1.34	1.28	1.32	0.78	1.27					

The osmotic value of the Hoagland solution (H) amounted to 0.011–0.013 M.

rate of exudation by the change in the osmotic value of the medium (see Material and methods).

In experiment 1 isotonic solutions were used so that we cannot calculate k . Afterwards, however, the rate of exudation of this plant was determined at different times under various conditions. Then the plant was placed on a more concentrated solution for a few minutes so as to obtain the data necessary for the calculation of k . The values found in this way follow below.

Medium	Min. after the change of the medium	Rate of exudation	k
Hoagland sol. (0.011 M)		20.4 mm/30 sec.	457
Mannitol sol. (0.012 M)	5	17.4 mm/30 sec.	463
Mannitol sol. (0.012 M)	30	14.6 mm/30 sec.	443
Mannitol sol. (0.012 M)	40	13.8 mm/30 sec.	423
Hoagland sol. (0.011 M)	5	17.4 mm/30 sec.	423
Hoagland sol. (0.011 M)	30	19.4 mm/30 sec.	423

Five minutes after the outer solutions had been changed no alteration in the value of k was yet perceptible, although the rate of exudation had already considerably changed at that time.

It appears from experiment 3 too, that k is not changed to any considerable extent by the lack of salts in the medium. When considering the values of k we must reckon with the influence of the osmotic value of the medium on this factor. The latter phenomenon, which had already been observed by ARISZ, HELDER and VAN NIE, is demonstrated in table 3. For this reason values of k can be compared only when solutions of the same osmotic value have been used. In experiment 3 the rate of exudation increased suddenly by 7.8 mm/30 sec when a solution of salts and mannitol was replaced by a Hoagland solution (third period) and when a solution of mannitol was changed for a Hoagland solution the sudden increase amounted to 7.4 mm/30 sec. We can conclude from these data that the values of

TABLE 3
Relation between the osmotic value of the medium and k

experiment	medium	H(0.013 M)	H + M (0.037 M)	H(0.013 M)	
4d	k	516	432	516	
	medium	H(0.012 M)	H + M (0.033 M)	H(0.012 M)	H + Glucose (0.032 M)
15c	k	411	368	433	356

k in the second and fourth period are only slightly different the change in the osmotic value of the medium being nearly the same in both cases, while the rates of exudation in the condition of equilibrium differed by 5 mm/30 sec.

In some experiments k certainly changed. In these cases however the rate of exudation was proved to be different under similar con-

ditions at various times. For this reason these changes are rather to be considered as phenomena of periodicity than as changes of the water conductivity caused by the presence or absence of salts in the medium. Periodical changes of k will be discussed in section 7.

In this case it appears that alterations in the factor k can be left out of consideration as the cause of changes in the rate of exudation. The other possibility mentioned was, that the rate of exudation would be altered as the result of a change in the osmotic difference between the solution in the vessels and the medium.

The osmotic value of the sap depends on the relation between the transport of water and the transfer of salts to the vessels. The water transport is directly dependent on the osmotic value of the medium; the transport of salts is influenced by the salt concentration of the outer solution (table 1 and 2).

The osmotic value of the outer solution remained unaltered in experiment 1, while the salt concentration was lowered. In this case less salt is given off to the vessels, while the amount of water transported there does not immediately change. The sap in the vessels is diluted and the osmotic value must decrease. Consequently the difference between the osmotic value of the sap and that of the outer solution is diminished and so the rate of exudation now falls too. In table 1 we can see, that under such conditions the osmotic value of the sap actually decreased.

In the other experiments the osmotic value of the medium was enhanced and in some the salt concentration was at the same time lowered. An increase of O_m in the formula $b_s = k(O_b - O_m)$ means a reduction of b_s , that is of the transport of water. The osmotic value of the sap should now increase if the salt secretion were to continue at the same rate. This has been found experimentally when the salt concentration of the outer solution was not lowered (experiment 3, second period; experiment 4, second and eighth period). When however less salt is given off, it is the relation between the decrease in the transport of water and the reduction of the salt secretion that controls the change in the osmotic value of the sap. In experiments 3, 4 and 5 the salt concentration of the medium has been lowered to nought every time, so the salt secretion should always decrease to the same extent. A diluted solution of mannitol would only slightly reduce the water transport, $O_b - O_m$ changing but little. As the salt secretion is reduced considerably, the sap in the vessels will be diluted as in experiment 1, in which the osmotic value of the medium was not altered at all. The rate of exudation slows down as a result of this decrease in the osmotic value of the sap. Salt is carried off from the vessels more slowly now. At last a new equilibrium is established between the rate at which salt is given off to the vessels and that at which it is removed by the flow of water. The osmotic value of the sap does not alter any more and so the rate of exudation is constant too.

Addition of a stronger mannitol solution causes a greater decrease of the water transport. In this case salt that is given off to the vessels is carried off very slowly. It is possible now, that less salt is removed

per unit of time than has been given off to the vessels and the sap must become more concentrated. In consequence more water is taken up and the transport of salts from the vessels is speeded up till an equilibrium has been attained. Here we meet a case analogous to that when only the osmotic value of the medium is changed: in both circumstances the transport of water is reduced as compared with the salt secretion.

The different effect caused by weak and more concentrated solutions of mannitol has been shown in fig. 4 and 5. The changes in the rate of exudation that are caused by a mannitol solution of moderate concentration can be explained in the same way: now the transport of water is reduced by the enhancement of the osmotic value of the medium to the same extent as the salt secretion is reduced because of the lack of salts in the outer solution. The rate of exudation must decrease, but the relation between water and salt transport is not altered and the osmotic value of the sap remains the same. The rate of exudation will neither increase nor decrease after the sudden change.

It will be clear that the changes in the rate of exudation resulting from a decrease in the osmotic value and a simultaneous increase in the salt concentration of the medium can be explained in a similar way. In that case more water is given off to the vessels — $O_b - O_m$ increases — as well as more salt. The flow of water becomes greater as the decrease in the osmotic value in the medium is made greater. The sap will become more dilute or more concentrated according to whether the increase in water transport is more or less than the increase in the salt secretion. Thus after the sudden rise the rate of exudation will either decrease or show a further increase.

It will seldom happen that water transport and salt secretion change to the same extent — which would mean a sudden change only — as this only occurs at one certain osmotic value of the medium. What value this is depends on the salt secretion and the value of k , and these are different for each plant.

A simultaneous decrease in the osmotic value and the salt concentration of the medium must cause an increase in the water transport, but also a reduction of the salt secretion. In consequence the sap is diluted, and after the initial increase the rate of exudation decreases. As a result the exudation changes in the same way as when only the osmotic value of the medium has been lowered. This is demonstrated in fig. 3 (sixth period) at the replacement of a Hoagland solution by distilled water.

Thus we can explain the changes in the rate of exudation caused by changes in the osmotic value and the salt concentration of the medium as the result of changes in water transport and salt secretion. In addition to the osmotic effect of the outer solution, a salt effect can be observed.

If the salt concentration of the medium is lowered while the osmotic value is not much enhanced, the osmotic value of the sap should decrease, as the solution in the vessels becomes more dilute. It has

been pointed out already that in such cases the osmotic value of the sap usually rose, though the increase was smaller when the outer solution contained mannitol alone than when it contained both mannitol and salt (table 2). An actual decrease of the osmotic value has been found only when the salt concentration of the medium was lowered without an enhancement of its osmotic value (table 1).

Conversely the osmotic value of the sap showed a decrease instead of an increase when a weak solution of mannitol was changed for a Hoagland solution.

More concentrated solutions of mannitol caused an increase of the osmotic value of the sap, just as was to be expected.

In our speculation we supposed k to remain unaltered. Now it has been pointed out already that k diminishes if the osmotic value of the medium increases. This could perhaps be the cause of the unexpected increase in the osmotic value of the sap, the water transport being impeded by a decrease of k . When b and k are known we can calculate what osmotic value the sap in the vessels of the roots should have. These values are stated in table 2 (O_b theor.). The increase in the osmotic value of the sap might be explained in this way in a few cases; experiment 3 for instance, where the differences between the values found and those that have been calculated do not exceed the error of the determination.

This deviation from the theory is demonstrated more clearly in experiment 4*d*. On a Hoagland solution the osmotic value of the sap was 0.048 M and on a solution of mannitol 0.059 M (fourth period) so that there was a considerable increase. Osmotic values of 0.071 M and 0.067 M would be expected according to the calculation; that is a decrease.

ARISZ, HELDER and VAN NIE also found a difference between the osmotic value of the exudation sap and the value that had been calculated from the formula. The former was too small. The same thing can be noticed in the example given above (experiment 4*d*). ARISZ, HELDER and VAN NIE mentioned several explanations for this phenomenon, which will be discussed more extensively later. We must state however, that the osmotic value of the exudation sap can also be higher than is to be expected. Thus in experiment 4 (table 2) an osmotic value of 0.049 M was found (fourth period), a value of 0.037 M being calculated. The same thing occurs in experiment 3, but then as has been pointed out already the difference is very small.

Of course experimental errors could be involved. An error can be made in the determination of the osmotic value of the sap as well as in the calculation of k , which is based on the determination of the osmotic value of the outer solutions. However it is improbable that this would explain the difference in experiment 4, the error being only 0.001–0.002 M.

We have pointed to the fact, that it takes some time before changes in the osmotic value of the sap in the roots are shown in the out-flowing sap. In experiment 4*a* the fact that the osmotic value of the sap was 0.061 M (eighth period) while the osmotic value of the medium

was 0.073 M, might be explained in this way. But this "retardation" can only account for too low a value after an increase of the osmotic value of the medium, or too high a value when the osmotic value of the medium has been decreased. So we must conclude that in the case of a small increase of the osmotic value of the medium and a decrease in its salt concentration the osmotic value of the outflowing sap can differ from the value calculated from the formula in two ways: the osmotic value changes in a direction opposite to our expectation, and its absolute value can become too high.

The changes in the rate of exudation observed under conditions as set forth above can be explained if we consider the action of the root as that of an osmometer. This working hypothesis, however, cannot account for all phenomena.

We have demonstrated that the decrease of the rate of exudation in the case of a decrease of the salt concentration of the medium, should be considered as the result of the fact that less salt is given off to the vessels. The rate of exudation shows a decrease within one or two minutes after the change of the medium; the same holds true for the increase when the salt concentration of the outer solution is enhanced. It follows from this that the salt secretion changes as soon as the salt concentration of the medium is altered. In experiment 3 the rate of exudation decreased from 19.4 to 11.2 mm/30 sec after an enhancement of the osmotic value of the medium from 0.013 M to 0.027 M. A more gradual decrease followed. This shows that the salt secretion must have diminished to a larger extent than the transport of water. As the latter was reduced by almost 50 %, it must be concluded that the salt secretion decreased to at least one half. The fact that in spite of the large sudden reduction of the water flow a further gradual decrease followed, indicates that considerably less salt was given off to the vessels immediately after the change of the outer solutions. Considering the magnitude of the salt secretion at the equilibrium, we must conclude that almost the whole reduction in the salt secretion took place within one minute after the salt had been removed from the outer solution. Likewise much more salt is apparently given off to the vessels as soon as salt can be taken up from the medium.

Thus the secretion of salt to the vessels is partially held up when no salt can be taken up from the medium. However some salt is still given off and the exudation continues, although at a smaller rate. The necessary salt must come from the tissue. This creates the impression that the process we called "salt secretion" consists of two components. Salt is continually carried from the tissue to the vessels, even when no salt is taken up from the medium. In addition salt taken up from the outer solution can be transported rapidly, and so probably directly, to the vessels. This part of the salt secretion, depending on the salt uptake, will henceforth be called "uptake secretion"; the other part is called "tissue secretion". We may observe that if the expression salt secretion is used, the whole process is meant that achieves the transfer of salts, from the medium or from the tissue, to

the vessels. The expression may be not altogether correct, but is used for brevity's sake.

The tissue secretion causes some transport of water. When the salt secretion is increased in conditions favourable for salt uptake, it is accompanied by an increase in the exudation: the extra exudation could be called "uptake exudation" to distinguish it from the "tissue exudation". It should be understood, however, that these expressions refer to the cause of the transport of water; in both cases part of the water at least comes from the medium.

§ 2. RELATION BETWEEN SALT CONDITION OF THE PLANT AND SALT SECRETION INTO THE VESSELS

It has been demonstrated in the preceding section that salt can be given off to the vessels even when salt uptake is not possible. This points to the fact that salt is transported from the tissue to the vessels.

Low salt plants are known to have a weak exudation (BROYER, 1951). Barley plants did not show guttation in such conditions. This indicates how important for exudation the salt condition of the roots is.

When a plant had been on distilled water for a day, both salt secretion and exudation were considerably reduced. It seems reasonable to attribute this to the depletion of salts in the root cells. In that case an increase of the salt secretion should be expected when the roots have the opportunity to improve their salt condition by accumulating salts from the medium. This is shown in experiment 6 (fig. 6). A plant was put on a weak salt solution after having been on a solution of mannitol during the previous night (a solution of mannitol was used instead of distilled water to avoid changes in the osmotic value of the medium). The rate of exudation and the salt secretion both increased. Substitution of a solution of mannitol for the salt solution caused a reduction of the exudation and salt secretion, but in the condition of equilibrium both were greater than before. A considerable increase was caused by a stronger salt solution (2 H). When the salts were again removed from the medium, neither

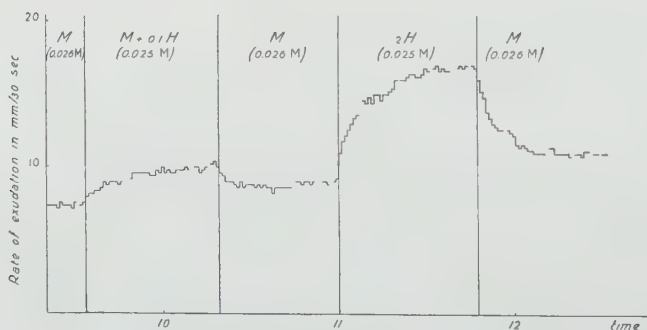


Fig. 6. Increase in the rate of exudation caused by a temporary addition of salt to the outer solution.

did salt secretion nor rate of exudation decrease to the same value as before, although of course some decrease could be observed. The salt secretion increased in this experiment from 0.31 to 0.56, that is by approximately 80 %. From this it can be concluded that the salt condition of the roots must have improved during the periods when salts could be taken up from the medium.

The increase of the salt secretion when salts can be taken up from the medium, can apparently be the result of a direct transport of salts to the vessels as well as of an increased tissue secretion. The pretreatment of the plant appears to be of importance for the tissue secretion. In this experiment however, the uptake of salts had been inhibited for a long time (about 16 hours). In most experiments plants remained on a solution without salts for half an hour only, so that the salt condition of the roots will have been altered less.

§ 3. INFLUENCE OF THE COMPOSITION OF THE MEDIUM ON THE EXUDATION

a. Relation between the composition of the medium and the rate of exudation

The rate of exudation proved to be stimulated by the presence of salts in the medium. It may be asked which salts are concerned here. Only meagre data on the influence of various ions on the exudation can be found in literature. In several cases solutions were used containing a mixture of salts; sometimes the different osmotic effect of different salt solutions has been left out of the reckoning. Comparing the exudation of wheat plants on solutions of KNO_3 , KH_2PO_4 and KHCO_3 with that of plants on distilled water, LUNDEGÅRDH (1945) found a decrease of the rate of exudation with however an increase in the concentration of the sap in the first two cases. He concluded from this that nitrate would impede the exudation. The presence of the other salts did not seem to have much influence on the exudation. RALEIGH (1946) investigated the influence of salts on guttation. Nitrogen, phosphorus, magnesium, calcium and potassium deficient tomato plants showed no guttation, but guttation could be evoked by adding respectively nitrate, phosphate or potassium to the medium. Addition of calcium or magnesium had no effect. In these experiments however, plants were used which had been grown in solutions lacking one or other element till deficiency symptoms were evident.

To gain an impression of the importance of various anions the exudation was studied when a complete Hoagland solution was replaced by a solution lacking nitrate, phosphate or sulfate. In the first case rate of exudation and salt secretion decreased in the same way as has been shown in fig. 1. In the other cases no change was observed.

These results indicate that nitrates in particular can stimulate the salt secretion. This conclusion can be confirmed by experiments in which the influence of different solutions of single salts was investigated. The concentration of the salts was the same every time. The

solutions had been made isotonic by adding mannitol if necessary. Changing a 0.005 M solution of KNO_3 for a solution of mannitol caused a decrease of the rate of exudation. The rate of exudation was likewise decreased when a solution of KHCO_3 or KH_2PO_4 was given instead of the KNO_3 solution. At equilibrium the same level was attained whether the outer solution contained mannitol, phosphate or bicarbonate (fig. 7). The presence of phosphate and bicarbonate seems to have no influence on the exudation, at least in brief

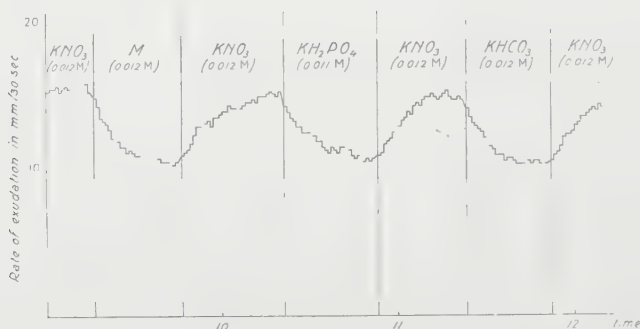


Fig. 7. Changes in the rate of exudation resulting from changes in the composition of the medium.

experiments. The osmotic value of the sap was 0.046 M when the medium consisted of a nitrate solution but 0.040 M and 0.042 M when mannitol and phosphate were present in the outer solution. This means, that in these cases the salt secretion was decreased.

Conversely an immediate increase in the rate of exudation could be observed when a plant was transferred to a solution of potassium nitrate after having been on an isotonic solution of mannitol. If instead of potassium nitrate potassium phosphate was used nothing happened (experiment 8, fig. 8). Potassium chloride could cause an

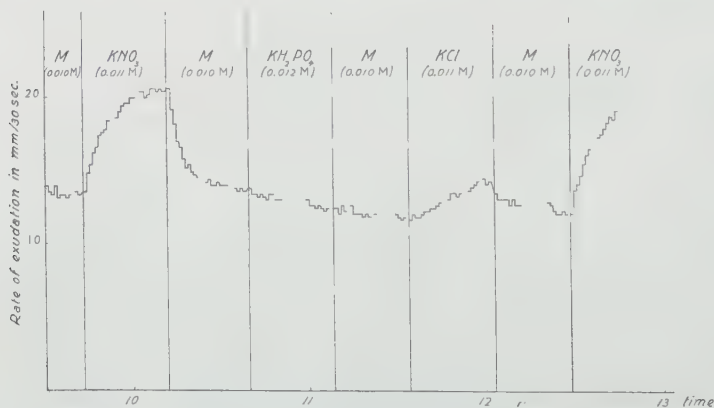


Fig. 8. Increase in the rate of exudation caused by adding KNO_3 or KCl to the medium.

increase in the exudation, but not to such an extent as the nitrate. Even when the roots had been in a solution of potassium chloride for some hours or for a whole night, the exudation did not attain the same rate as when nitrate was present in the medium. A salt effect seems to be caused only by the presence of nitrate or chloride in the outer solution.

We must now investigate to what extent cations are of importance. From the data on water uptake we should expect that potassium and calcium especially would have some influence. It has been stated that in general K promotes the uptake of water, while Ca hampers this process. Most of these results, however, have been obtained from experiments of several days, during which plants were grown under different conditions of salt supply. In these experiments secondary phenomena such as differences of development can be of importance (KISSER, 1927; GASZNER and GOEZE, 1934). However TAGAWA (1934) also demonstrated the influence of K and Ca in a shorter time: decapitated plants of *Phaseolus*, as well as intact ones, took up more water when the outer solution contained much potassium and little calcium than they did in the reverse case.

SPEIDEL (1939) found a stronger exudation with plants grown in solutions containing little potassium and much calcium than with plants cultivated in conditions of relative Ca deficiency. He supposed that the root cells would give off less water as the result of the swelling of the protoplasm under the influence of potassium. It is known from experiments with intact plants that potassium not only promotes the uptake of water, but diminishes the transpiration as well. LUNDEGÅRDH (1943, 1945) also found a retardation of the rate of exudation if potassium was present whereas calcium had a favourable effect. The differences, however, seem to be small.

We should thus expect that potassium and calcium would perhaps affect not so much the salt secretion as the permeability of the roots for water, which should appear as changes in the factor k .

In table 4 values of the rate of exudation and salt secretion are given, which were obtained when the plants had been on solutions of potassium, calcium, ammonium, or sodium nitrate for half an hour. These values are much the same. No sudden change in the rate of exudation resulting from a change of the medium has been found, so long as the nitrate concentration remains unaltered. Sometimes after a longer period a gradual decrease in the rate of exudation could be observed. This decrease, however, was not the result of any particular change of the medium since it continued during the whole experiment. It might be attributed to the use of an unbalanced salt solution. The same thing could be seen in experiments on the influence of various anions. In most experiments a complete Hoagland solution has been used for this reason, although the presence of nitrate appeared sufficient to cause the salt effect.

The chlorides of various elements were also proved to have the same effect on the exudation (table 4).

It seems that the salt secretion is not much influenced by the nature

of the cations present in the medium. As neither the rate of exudation nor the osmotic value of the sap were changed when a solution of a potassium salt was replaced by a solution of a salt of calcium, sodium or ammonium, we can deduce that k would not change much either. Indeed k was proved to remain unaltered under these conditions.

TABLE 4

Rate of exudation and salt secretion in equilibrium on solutions containing different cations. The solutions contained 0.005 M salt and were isotonic.

Expe- riment	Medium	KNO ₃	NaNO ₃	KNO ₃	NH ₄ NO ₃	KNO ₃	Ca(NO ₃) ₂
9. . . .	<i>b</i>	10.6	10.6	9.8	8.6	8.4	8.6
	S	0.46	0.42	0.39	0.33	0.33	0.33
	Medium	KNO ₃	Ca(NO ₃) ₂	KNO ₃	Ca(NO ₃) ₂		
9a. . . .	<i>b</i>	16.6	16.4	15.0	14.6		
	S	0.75	0.73	0.60	0.57		
	Medium	KNO ₃	NaNO ₃	KNO ₃	KCl	KNO ₃	NH ₄ NO ₃
9b. . . .	<i>b</i>	11.4	12.2	12.4	10.6	12.0	11.8
	S	0.48	0.52	0.48	0.40	0.46	0.44
	Medium	KCl	CaCl ₂	KCl	NH ₄ Cl	NaCl	KCl
9c. . . .	<i>b</i>	8.4	7.8	8.0	7.0	7.2	7.4

Even when a plant had been for twenty four hours on a solution containing only potassium nitrate or calcium nitrate, nothing happened when this solution was replaced by the opposite one of calcium or potassium nitrate respectively. In similar experiments, however, there is the difficulty that the exudation can change spontaneously over such a long period.

b. Concentration and composition of the exudation sap in relation to the composition of the medium

Different opinions exist concerning the relation between the concentration of the sap and that of the medium. According to SABININ (1925) the concentration of ions in the sap is independent of their concentration in the outer solution. The amount of every ion present in the sap should be characteristic for that ion. Thus he found potassium and phosphate in the sap in higher concentrations than in the outer solution, while calcium and ammonium were found in lower concentrations. LAINE (1934) on the contrary stated that the concentrations of cations (potassium, calcium and magnesium) in the sap was proportional to their concentration in the medium: $s = kc^{1/n}$, s being the concentration of the ion in the sap, c that in the medium and k and n constants. LUNDEGÅRDH (1943, 1945) found that an increase in the concentration of potassium nitrate in the outer solution caused a large increase in the concentration of potassium as well as of nitrate in the sap. When the outer solution contained calcium nitrate the salt concentration of the sap was only slightly increased, when the concentration of the medium was enhanced. The rate of exudation decreased because the salt concentration of the outer solution was greater so that in this case the amount of salt given off to the sap

did not alter. If the solution contained potassium nitrate actually more salt was given off as the salt concentration increased. LUNDEGÅRDH did not therefore find any definite relation between the concentration of a salt in the medium and that in the sap, although some influence could be demonstrated.

On account of the influence of the osmotic value of the medium on that of the sap, which has been demonstrated in section 1, it is necessary to use isotonic solutions when investigating the relation between the salt concentration of the medium and that of the sap. LAINE's solutions were not isotonic but their concentrations were rather low, so that their osmotic effect may have been small. In LUNDEGÅRDH's experiments solutions of different concentrations had also different osmotic values; LUNDEGÅRDH, however, considers the osmotic effect of the medium on the sap concentration to be of no importance. It is difficult to evaluate these results.

Even if we eliminate the osmotic effect of different solutions some difficulties arise. As we argued before, the concentration of the sap depends on the relation between the transport of water and the transfer of salts to the vessels. The first is, among other things, influenced by k , a factor that differs with each plant. The transfer of salts is influenced by changes in the salt concentration of the medium, as has been pointed out in section 1, but it also depends on the salt condition of the roots. Moreover the concentration of the sap was not the only factor found to change when the salt secretion was altered, for the amount of sap given off to the vessels per unit of time also varied. This means a change in the rate of water transport, which must again influence the salt concentration in its turn. Finally the salt secretion is influenced not only by the salt concentration of the outer solution but also by its osmotic value. It will be clear that so many factors can influence the concentration of the sap, that the possibility of representing the relation between its salt concentration and that of the medium by a simple formula is as LUNDEGÅRDH (1945) has already stated, hardly to be expected.

We have seen that one part of the salt secretion depends on the possibility of taking up salts from the medium. As the latter process will depend on the concentration of the salts in the medium, it might be expected that some relation would be found between the concentration of the medium and this part of the salt secretion. For reasons mentioned above, this concerns not changes in the concentration of the sap but changes in the amount of salt given off to the vessels per unit of time.

In table 5 the results are to be seen of some experiments done to determine this point. The part of the salt secretion depending on the uptake of salts from the medium is calculated by subtracting the amount of salt given off when no salt is available in the medium, from the salt secretion after the addition of salt to the outer solution. Only in one case could any regularity be observed in the increase of the uptake secretion with the increase of the salt concentration of the medium. Irregularities can be attributed to several factors. The

most important is the periodicity of salt secretion. It appears that the tissue secretion changes during the day. For a discussion of this phenomenon readers are referred to section 7. If we therefore calculate the uptake secretion by subtracting the tissue secretion found at one time from the whole amount of salt given off at another time, incorrect

TABLE 5

Relation between the salt concentration of the outer solution and the salt secretion (on isotonic solutions)

Medium	Salt secretion	in mm ³ × M/30 sec.	
	experiment 2	2a	2b
Hoagland 100 %	1.26	1.04	1.01
„ 0 %	0.65	0.90	0.61
„ 10 %	0.90	—	0.66
„ 20 %	1.04	1.01	0.83
„ 30 %	—	—	0.58
„ 50 %	1.16	1.08	0.77
„ 200 %	—	1.22	—
„ 100 %	1.35	—	0.72

values will be obtained. To obtain reliable osmotic values for the sap it is necessary that the plants shall be on a certain solution for at least half an hour, so that the influence of so few concentrations can be investigated that it is impossible to draw any conclusions as to the relation between the salt concentration of the medium and the amount of salt given off to the vessels.

The importance for the exudation of the presence of nitrate ions in the medium leads to the supposition that they should compose an important part of the ions in the sap. LUNDEGÅRDH (1943, 1945) found indeed mainly potassium nitrate in the sap of wheat plants. Cl, PO₄, SO₄, NH₄ and Ca were present in very small amounts. Organic substances have seldom been found in the sap of herbaceous plants. Large amounts of sugar such as appear in the sap of various trees do not seem to occur (VAN OVERBEEK, 1942; SKOOG, BROYER and GROSSENBACHER, 1938). LITVINOV (according to LAINE) only, mentions the presence of relatively large amounts of organic acids and organic nitrogen compounds in the exudation sap of pumpkins.

Much nitrate was found in the exudation sap of tomato plants. The presence of phosphate, carbonate, or bicarbonate, and a very little sulfate could also be demonstrated. Chloride was found only when the plant was on a solution of chloride, while the other ions were also found in the sap of plants that had been on distilled water for some time.

Potassium as well as calcium and ammonium seemed always to be present, potassium probably predominating.

We did not succeed in demonstrating the presence of sugars or amino acids.

Approximately the same results were found with plants of *Sanchezia nobilis*, but the sap of this plant seemed to contain more chloride; that is to say that chloride could also be demonstrated in

the sap of plants that had been grown on a Hoagland solution made with tap water, which contains always some chloride.

The results of several determinations of the concentrations of nitrate and phosphate in the sap are summarized in table 6. These determinations were made at different times of the day (the salt secretion changes during the day as will be shown later) and in different circumstances so that the roots were on isotonic solutions with or without salts. Sap was collected during periods of approximately two hours. The amount of sap was too small to determine the concentration of other ions beside nitrate and phosphate. It is not, however, to be expected that other ions were of much importance. From a comparison of the osmotic value of the sap and its nitrate concentration it appears that other anions could only have been present in very small amounts, while we found earlier that the various cations are not of great importance for the exudation.

The nitrate concentration of the sap always exceeded that of the Hoagland solution. By the evening less nitrate was given off to the vessels while the exudation of sap likewise decreased. Next morning both nitrate and sap were given off again in greater amounts.

The sap contained much more nitrate than phosphate. The secretion of phosphate into the vessels changed proportionately more during the day than the nitrate secretion, but the changes usually coincided. Like LUNDEGÅRDH we found that under these conditions the sap consists chiefly of a solution of nitrate, probably potassium nitrate.

When a plant was transferred from a Hoagland solution to an isotonic solution of mannitol less sap was exuded and less nitrate was given off to the vessels. Nitrate however remained the main constituent of the sap even when no salt could be taken up from the medium. When decapitated plants had been on a mannitol solution or distilled water for 36 hours the amount of nitrate given off to the vessels per hour might still be approximately one third of the quantity secreted while the plant was on a Hoagland solution.

If a solution without salts was replaced by a Hoagland solution more nitrate was given off to the vessels and the exudation of sap also increased.

The quantity of phosphate in the sap appeared to be influenced less by changes of the medium. Sometimes even more phosphate was given off while the plants were on a mannitol solution or distilled water for 2-4 hours, than during periods in which phosphate could be taken up (table 6). This could lead to a considerable increase in the phosphate concentration of the sap, the rate of exudation being diminished as a result of the smaller nitrate secretion. Conversely the secretion of phosphate into the vessels did not always increase when phosphate could be taken up from the outer solution as was the case with nitrate. Also when the uptake of salt was inhibited for a longer time the secretion of phosphate was not so much affected as that of nitrate. The sap of a plant which had been on distilled water for twenty four hours after decapitation contained about three quarters

TABLE 6
Quantity of sap and amounts of nitrate and phosphate given off during two days on a Hoagland solution or an isotonic solution lacking salt

Experiment 10. Tomato plant							
time	medium	g. sap/hour	osmotic value sap	concentration of nitrate	amount of nitrate m.e./hour	concentration of phosphate	amount of phosphate m.e./hour
8.15-10.15	Hoagland	2.539	0.049 M	0.024 M	0.061	0.0010 M	0.0026
10.15-12.15	Hoagland	2.722	0.051 M	0.023 M	0.063	0.0016 M	0.0044
12.15-14.15	Hoagland	2.810	0.050 M	0.022 M	0.062	0.0016 M	0.0046
14.15-16.15	Hoagland	2.666	0.047 M	0.022 M	0.059	0.0017 M	0.0046
16.15-18.15	Hoagland	2.682	0.045 M	0.019 M	0.051	0.0018 M	0.0049
18.15- 8.15	Hoagland	1.945	0.034 M	0.015 ⁵ M	0.030	0.0012 M	0.0023
8.15-10.15	Hoagland	2.449	0.034 M	0.017 M	0.042	0.0011 M	0.0026
10.15-12.15	Mannitol	1.331	0.034 M	0.018 M	0.024	0.0018 M	0.0024
12.15-14.15	Mannitol	1.143	0.035 M	0.014 ⁵ M	0.017	0.0015 M	0.0017
14.15-16.15	Hoagland	2.045	0.033 M	0.017 ⁵ M	0.036	0.0012 ⁵ M	0.0026
Experiment 10a. Sanchezia nobilis							
8.15-10.15	Hoagland	3.888	0.033 M	0.018 M	0.068	0.0004 M	0.0016
10.15-12.15	Hoagland	3.992	0.034 M	0.018 M	0.072	0.0004 M	0.0016
12.15-14.15	Hoagland	4.010	0.033 M	0.018 M	0.071	0.0004 M	0.0016
14.15-16.15	Hoagland	3.990	0.032 M	0.015 M	0.060	0.0004 M	0.0017
16.15-18.15	Hoagland	4.036	0.032 M	0.014 M	0.057	0.0003 M	0.0011
18.15- 8.15	Hoagland	4.200	0.025 M	0.014 M	0.057	0.0002 M	0.0008
8.15-10.15	Hoagland	4.058	0.030 M	0.015 M	0.061	0.0003 M	0.0011
10.15-12.15	Hoagland	3.934	0.031 M	0.016 M	0.063	0.0002 M	0.0012
12.15-14.15	Mannitol	2.005	0.027 M	0.012 M	0.023	0.0007 M	0.0014
14.15-16.15	Mannitol	1.843	0.021 M	0.011 M	0.020	0.0009 M	0.0017
16.15-18.15	Hoagland	3.540	0.027 M	0.013 M	0.056	0.0004 M	0.0015

the amount of phosphate that had been given off on a Hoagland solution, while the quantity of nitrate was decreased to one third.

These data are for the sap of tomato plants (table 6, experiment 10) as well as that of *Sanchezia nobilis* (table 6, experiment 10a).

Fig. 9 also demonstrates the great influence of the uptake of salts on the secretion of nitrate into the vessels. In these experiments solutions of 0.005 M KNO_3 , KH_2PO_4 , KCl or a solution of mannitol were used. A small quantity of CaSO_4 was added to the first three solutions to counteract an eventual injurious effect from the potassium ions. The solutions were isotonic.

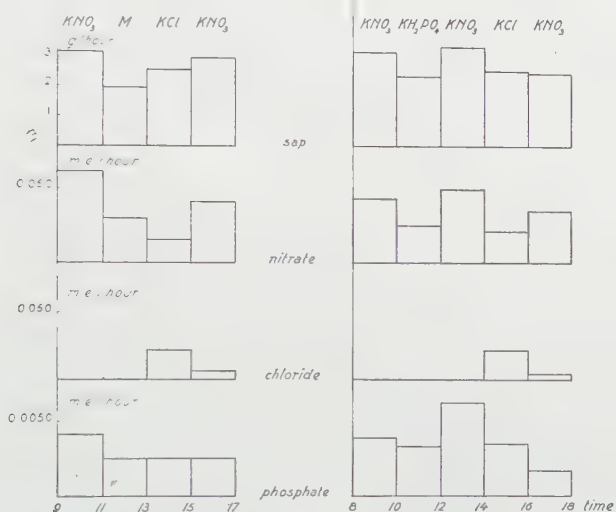


Fig. 9. Comparison of the amounts of sap, nitrate, chloride and phosphate given off on isotonic solutions containing KNO_3 , KCl, KH_2PO_4 or mannitol.

Exudation of sap and secretion of nitrate decreased considerably every time the plants were transferred to a solution lacking nitrates. Addition of nitrate to the medium caused an increase of both.

When the plants could take up chloride from the medium, chloride was found in the sap in considerable quantity. This was accompanied by an increase in the exudation of sap as has already been demonstrated in fig. 8. This is seen most clearly when a solution lacking salts is replaced by a solution of potassium chloride.

The large decrease in secretion of chloride when potassium chloride was again removed from the medium is remarkable. It appears that chloride, like nitrate, can be transported directly to the vessels after having been taken up from the medium. It seems however, that only a small quantity of chloride goes from the tissue to the vessels. This suggests that chloride is present in the tissue in small quantities only, which would mean that the greater part of the chloride taken up from the outer solution is transported directly to the vessels. The results of VAN NIE (unpublished) are in favour of this supposition.

The amount of chloride present in the sap of plants which had been on distilled water for some days and had then been put on a solution of potassium chloride and decapitated, did not show any further increase after the first few hours. When a considerable amount of chloride had been transported to and accumulated in the tissue an increase of the tissue secretion and so of the whole salt secretion might have been expected as we have seen in experiment 6 (fig. 6). A similar case arises when a low salt plant is put on a solution of potassium nitrate: the amount of nitrate given off to the vessels and the exudation continue to increase for more than twenty four hours. Of course before this could be stated with certainty it would be necessary to compare uptake and secretion of chloride at the same time.

In experiment 11 (fig. 9) the amount of phosphate given off to the sap did not change when a solution of potassium nitrate was replaced by a solution of potassium phosphate during two hours. In the next period however when the phosphate was absent again from the medium, more phosphate was given off to the vessels. In another experiment, not recorded here, the same has been found; the secretion of phosphate this time increased after the substitution of a phosphate solution for a nitrate solution, but a further increase followed in the next period, when phosphate was absent from the medium. This indicates that while phosphate is taken up from the medium, it can take a rather long time before it reaches the vessels. The transport of phosphate to the vessels seems to be much slower than that of nitrate or chloride. This conclusion is consistent with the fact that phosphate could not stimulate the exudation when a mannitol solution was replaced by a solution of phosphate (fig. 8).

It seems that phosphate is transported to the vessels in some other way to nitrate and chloride. The small influence of the phosphate concentration of the medium on the amount of phosphate given off to the vessels may indicate that only the so-called tissue secretion transfers phosphate to the vessels. The secretion will then remain unaltered so long as sufficient phosphate is available in the tissue.

We have already concluded, from a comparison of the osmotic value of the sap with its nitrate concentration, that nitrate remains the main constituent of the sap when no salt uptake takes place. This was checked by determining the conductivity of the sap (nitrate being supposed to be present as potassium nitrate), the same conclusion being reached from the results.

To explain the gradual increase in the rate of exudation after the addition of mannitol to the medium EATON (1943) supposed mannitol to permeate into the roots. It has been pointed out already in the Introduction that this supposition appears to be superfluous. From the determinations of the salt concentration of the sap we must now conclude that there really is no evidence for the permeation of mannitol into the vessels. The increase in the osmotic value of the sap following a rise in the osmotic value of the medium is caused by an increase in its salt concentration.

In a few cases, however, the osmotic value of the sap did not con-

form to the nitrate concentration. When the osmotic value of the sap, which could be calculated from its nitrate content, was higher than the value that had actually been found, the differences were always small, so that they might be accounted for by experimental errors. It has been pointed out in the chapter Material and methods that the concentration of the sap calculated from the amount of nitrate and phosphate found may be somewhat too high. If we calculate the osmotic value of the sap from the amount of nitrate present, supposing all nitrate to exist as fully dissociated potassium nitrate, we shall obtain too high a value if in fact some of the nitrate is present as the calcium salt.

Several times the sap contained less nitrate than was to be expected from its osmotic value, especially when the plant had been for a longer time on a medium lacking salts (a solution of mannitol as well as distilled water). The difference could amount to one fourth of the total osmotic value (0.01 M boric acid), but it was usually not so large.

This would indicate that other substances can also be present. The presence of phosphate could not account for the difference. Electrolytes seem to be concerned as the conductivity of the sap was also greater than was to be expected from the nitrate concentration.

We have not succeeded in identifying this substance or substances. As carbon compounds were proved to be present, it seemed most plausible that the unknown substance would be carbonate, bicarbonate, or an organic acid. Sugar and amino acids are not present in demonstrable quantities as has been mentioned before. According to LUNDEGÅRDH (1945) no organic acids can be found in the sap of wheat plants, but bicarbonate can be demonstrated in considerable quantities. Under certain circumstances he found a shift of the relation between cations (K^+) and anions (NO_3^-) resulting from a shortage of the latter. In his opinion bicarbonate would be compensating in this case. He confirmed this supposition by determinations of the bicarbonate concentration of the sap.

We have tried to gain an impression of the nature of the unknown substance by making titration curves of the sap.

The pH of the sap itself was between pH 5.5 and 6.0. It could rise to 6.8 after one or two days.

The titration curves were made in the following way: 15 ml of sap was titrated with diluted HCl, the pH having been adjusted first to 10.5–11 by adding NaOH. The results are to be seen in fig. 10, in which is represented the relation between the pH of the sap and the amount of dilute acid necessary to shift it by half a unit. The buffer capacity of the sap proved to be largest between pH 6 and 7 and pH 9 and 10. In all curves a maximum was found in those ranges. The buffer capacity was, however, very small between pH 3.5 and 5.5. Were organic acids, such as for instance malic acid, present a good buffering at low pH values would be expected. This was verified by making a titration curve after the addition of a small quantity of malic acid to the sap. In this case the sap was actually much more

buffered at low pH values. This points to the fact that if such acids are present, their quantity must be very small.

The buffering at pH 6-7 can be explained partially by the presence of phosphate. In fig. 10 next to the titration curve of the sap a titration curve of 15 ml of a phosphate solution of the same concentration is represented. This solution proves to be not so well buffered, which points to the presence of another substance. When a small amount

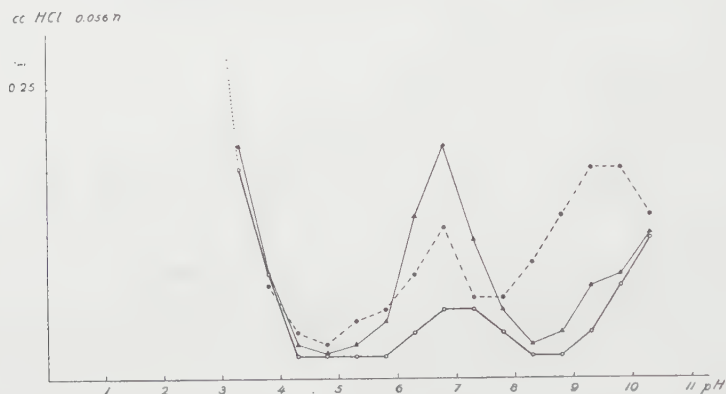


Fig. 10. Relation between the quantity of HCl required to shift the pH by half a unit and the pH. ●-----● sap; ○——○ solution of KH_2PO_4 containing as much phosphate as the sap; ▲——▲ solution of phosphate and 0.001 M. KHCO_3 .

of potassium bicarbonate (0.001 M.) was added to this solution, a stronger buffering was found between pH 6 and 7, stronger in fact than was shown by the sap itself. So it seems that the amount of bicarbonate present in the sap must be very small, and probably not sufficient to account for the difference between nitrate concentration and osmotic value.

c. Influence of the pH of the outer solution on the exudation

The exudation of wheat roots is not according to LUNDEGÅRDH (1949) very sensitive to changes of the pH of the medium; less sap was exuded only at very low (3) or high (9) pH values. There are few data on the influence of pH on water uptake by roots. However it is known from many experiments that the permeability to water of pieces of tissue can be influenced very much by changes in the pH of the outer solution. The results of these experiments are not always in accordance. This may be attributed partly to differences between the methods used and partly to the fact that different objects have been investigated (DRAWERT, 1952). The water permeability was usually found to be maximal at a not too acid pH and to decrease at lower and higher values (VON GUTTENBERG and MEINL, 1952).

The pH of the solutions used in the preceding experiments varied between 5.2 and 6.0, so we thought it important to investigate to

what extent changes in the pH of the medium could possibly be concerned with the observed changes of the rate of exudation. Special attention was paid to changes of k , although we must bear in mind that k , the conductivity of roots for water, need not be identical with the permeability to water of the pieces of tissue mentioned above. In the latter case the rate at which water goes from the medium into the vacuole is determined (transmeability according to ARISZ, 1945) while if intact roots are concerned, we investigate the rate at which water goes from the medium to the vessels, either passing through the cytoplasm or even through the cell walls.

The rate of exudation proved to decrease when a Hoagland solution (pH 5.2) was replaced by a similar solution whose pH had been increased to 7.2 or decreased to 3.4 by the addition of KOH or H₂SO₄. The decrease in the rate of exudation set in several minutes after the change of the pH. The exudation decreased continuously at about the same rate and even after one hour no constant level had been attained. The rate of exudation increased gradually when these solutions were replaced again by a Hoagland solution with a pH of 5.2.

Changes in the pH of the outer solution seemed to have little influence on the exudation so long as the value did not drop below 4.5 or rise above 6.5. More acid or alkaline solutions caused the exudation to decrease. This decrease was accompanied by a decrease of k . This can be seen in table 7. The salt secretion was not influenced much by the changes in the pH of the medium: as the rate of exudation decreased the osmotic value of the sap increased, sometimes to twice its initial value. The data in table 7 (experiment 13) indicate a small decrease of the salt secretion, but it may be questioned if this is a real decrease. At the moment the osmotic value of the sap was determined no equilibrium had been attained, the rate of exudation was still decreasing and so the osmotic value was probably rising. The osmotic value of the outflowing sap might have been lower than that of the sap in the vessels of the roots at the same time. In this way the value of the salt secretion would turn out to be too small.

TABLE 7

Influence of the pH of the medium on rate of exudation (b): osmotic value of the sap (O_b); salt secretion (S) and conductivity for water of the roots (k). All values were determined when the plants had stayed on a certain solution for one hour.

Medium	pH	b	O_b	S	k
Hoagland solution	5.20	9.4	0.043	0.40	170
" "	3.25	3.5	0.091	0.32	66
" "	5.20	9.1	0.047	0.43	166
" "	7.50	8.5	0.050	0.43	170

The decrease in the rate of exudation caused by changes in the pH of the medium was always completely reversible. The value of k increased again, while the osmotic value of the sap showed a decrease.

When a dilute acid or alkali was used, that is when no salts were present in the medium, the same results were found.

It is probable that the changes of the pH of the medium are of no

importance to the phenomena described in the preceding sections, as the differences of pH were rather small. It appears moreover that changes in the rate of exudation resulting from changes in pH follow another course to changes induced by alterations of the osmotic value or the salt content of the outer solutions.

§ 4. STIMULATION OF THE EXUDATION UNDER THE INFLUENCE OF SUGAR

The exudation of tomato plants has been shown to increase if the plants can utilize more sugar (WENT 1944). It has been pointed out already that an increase in the rate of exudation can have two causes; either the factor k must become greater, or a greater amount of osmotically active material must be given off to the vessels. In this case the first possibility would not seem very probable and indeed will prove to be unimportant.

LUNDEGÅRDH (1945) on the contrary found a decrease in the rate of exudation on the addition of glucose to the medium. Less salt was given off to the vessels; because in LUNDEGÅRDH's opinion more nitrate would be assimilated in the roots when more sugar was available.

WENT applied sugar to the plants by submerging two leaves left on the stump in a solution of sucrose. This method meets with some difficulties, as the effect of sucrose administered in this way is perceptible only after twelve hours. For this reason we added sugar to the medium. Using this technique WENT could find no effect, but on the other hand WHITE (1938) and STREET and LOWE (1950) have found that tomato roots are able to take up sucrose from a nutrient solution. It may be observed that in these experiments as in our own the medium consisted of a solution, whereas WENT used plants grown in soil which was sprinkled with a solution of sucrose.

The influence of sucrose was first investigated as it was known from the investigations just mentioned that it is taken up by the roots of tomato plants. Addition of sucrose to the outer solution, however, meant an increase in the osmotic value of the medium. To meet this difficulty rather low concentrations of sucrose were used. Moreover the exudation on a solution to which sucrose had been added was always compared with that on an isotonic solution containing mannitol instead of sucrose.

Experiment 14 (fig. 11). The Hoagland solution was first replaced by one that had been made isotonic with the solution containing sucrose, so that it would be possible to gain some idea of the changes due to a purely osmotic effect. When the plants had been on a Hoagland solution again for some time, sucrose was added, while salts were still present. At first the effect was the same as when a solution of mannitol was used: the rate of exudation fell suddenly and afterwards increased gradually. This effect must be considered purely osmotic. After approximately 20 minutes, at a time when equilibrium should have been attained, the rate of exudation showed a gradual increase, while after a good hour, approximately the same rate was attained as had previously been recorded on a Hoagland solution (whose

osmotic value is lower). The rate of exudation amounted to 27.2 mm/30 sec at this time, while it was only 13.4 mm/30 sec when the plant had stayed on a mannitol solution for an equal period.

When the solution containing sucrose was changed again for a Hoagland solution, the rate of exudation rose suddenly by a large amount — osmotic effect — but afterwards an unusual strong and prolonged decrease followed. After one hour no equilibrium had yet been attained, while as a rule this takes about fifteen minutes.

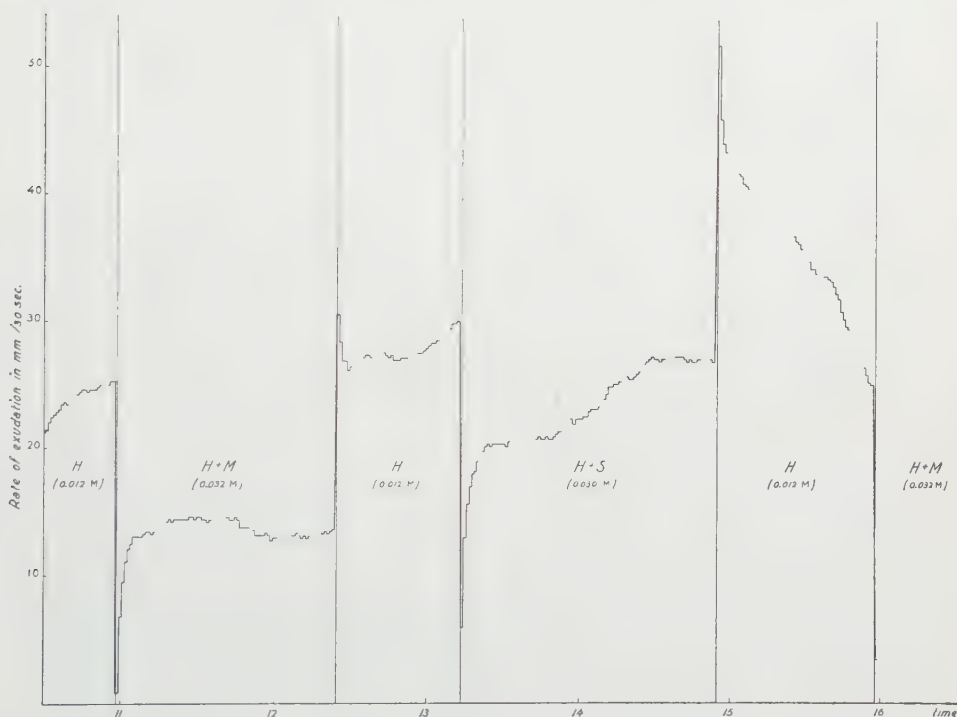


Fig. 11. Effect of adding sucrose (0.02 M.) to a Hoagland solution on the rate of exudation, compared with the effect of mannitol.

When the osmotic value of the medium was lowered the sudden rise in the rate of exudation was approximately the same whether sugar or mannitol had been used. This indicates that the stronger exudation in the presence of sugar cannot be attributed to a change of k . The osmotic value of the sap in the condition of equilibrium was 0.035 M when the medium consisted of a solution of salt and mannitol, and 0.038 M after a stay on the solution with sucrose. Although the difference was small, a greater amount of osmotically active substances must have been given off to the vessels in the latter case to cause the stronger exudation. Thus the salt secretion — or more in general the secretion of osmotically active substances — proves to be stimulated by the presence of sucrose in the medium.

osmotic value of the medium accompanied by an increase in its salt concentration. The gradual increase, however, was much greater than when the salt solution was substituted for a mannitol solution, whereas we should expect a smaller increase because of the periodicity. An equilibrium was not attained but a strong decrease in the rate of exudation set in after approximately 20 minutes. It seems plausible to attribute this phenomenon to the action of sugar taken up from the medium in the preceding period. This would mean that sugar acts as soon as salt can be taken up. At first the exudation is stimulated but as in experiment 14 the exudation must decrease again as the supply of sugar runs out.

It appears that the rate of exudation was not influenced by the sugar so long as the outer solution did not contain salts, nor was the secretion of osmotically active substances promoted. The results of

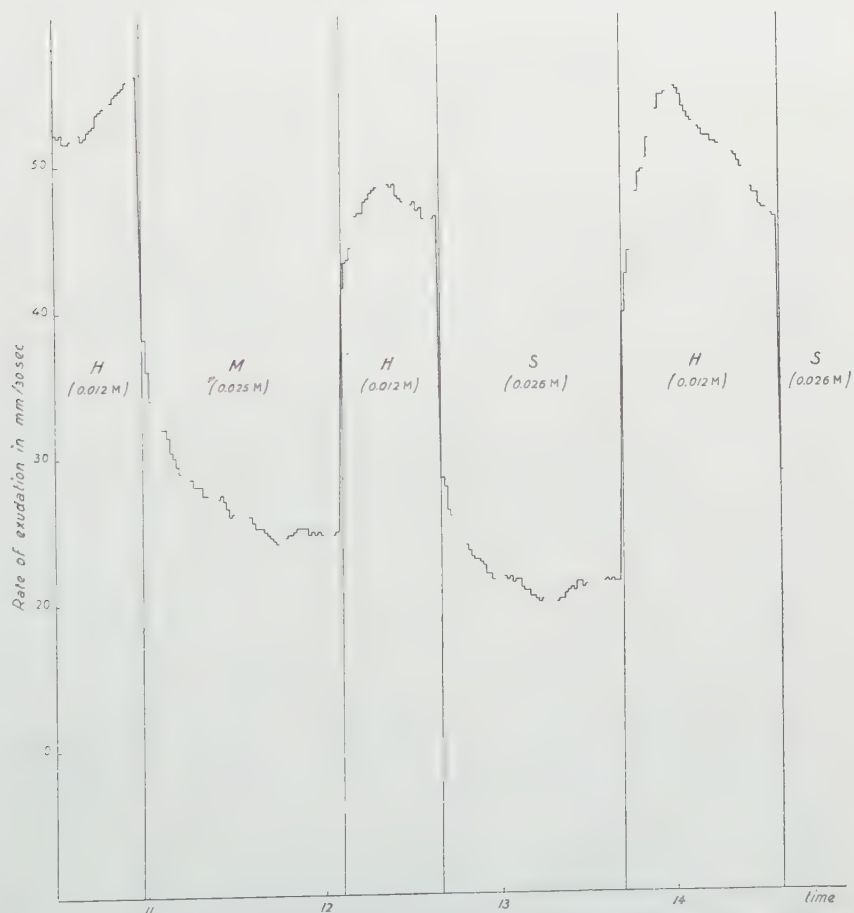


Fig. 12. Changes in the rate of exudation caused by replacing a salt solution by a solution of sucrose or mannitol, lacking salts.

two similar experiments have been recorded in table 9. We must emphasize that a stimulating effect of sucrose has only been found in cases when the outer solution contained both salt and sugar. This points to the fact that sucrose acts on the secretion of salts into the vessels. It is not probable that sucrose permeates into the vessels and is the cause of the stronger exudation, as this would also happen in the absence of salts. This was investigated by examining sap for the presence of sugars by means of the reaction with *α*-naphthol and sulfuric acid as well as by following the method of Hagedorn-Jensen. No sugar was found, even when the plants had been on a sucrose solution for several hours.

TABLE 9
Influence of sucrose on rate of exudation and salt secretion in solutions lacking salt as compared with the influence of mannitol

Experiment	Medium	H (0.015 M)	M (0.015 M)	H (0.015 M)	Sucrose (0.014 M)	H (0.015 M)
15a	<i>b</i>	21.6	15.2	23.4	14.4	25.6
	S	0.86	0.48	0.90	0.37	0.73
15b	Medium	H + M	M	M + Sucrose	H + Sucrose	
	<i>b</i>	20.6	7.0	3.2	22.6	

We did not succeed in demonstrating any stimulation by adding glucose to the medium.

The exudation of *Sanchezia* plants was not influenced very much by the addition of sucrose. Glucose could also exercise some influence. The sugars seemed to produce that effect only when salts were present in the medium, but because of the smallness of the stimulation it was difficult to state it with certainty.

That sucrose seemed to influence the exudation only when the outer solution contained salts beside the sugar was rather unexpected. First we must consider the possibility that the presence of salts is of importance for the uptake of sucrose. STREET and LOWE (1950) stated that the uptake of sucrose by the roots of tomato plants was favoured by the presence of phosphate. This would explain why we could not find an effect of sucrose when salts were lacking. It does not, however, seem very probable, that this is so because of the unusual course of the exudation when salts were added to the outer solution after the plant had been on a sucrose solution. This indicates as we explained above, that sucrose has been taken up while salts were absent.

It is difficult to explain this action of sucrose on the exudation. It might be that the uptake of salts from the medium is promoted. It is known that the salt uptake is related to the sugar condition of the roots. This has already been pointed out by HOAGLAND and BROYER (1936). STEWARD and PREVOT (1936) found that high salt barley roots could take up as much bromide as low salt, high sugar plants if glucose was added to the medium. It has been shown in several investigations with barley (HUMPHRIES 1951) as well as with maize (VAN ANDEL, ARISZ and HELDER 1950; HELDER 1952) that more phosphate was taken up if glucose was added to the medium.

According to HUMPHRIES the uptake of nitrate would also be stimulated. The last fact might be of importance here, as we should expect that an increased secretion of nitrate especially would be the cause of the increase of the exudation. In most investigations just mentioned however, it was the accumulation of ions in the root cells that was stimulated in particular, and we do not know if this also means an increase in the secretion of salts into the vessels. If we suppose that the increase in the exudation is caused by a stimulation of the salt uptake, then it seems curious that it takes so much time before this effect is noticeable, whereas the presence of salts always influences the exudation immediately. We will return to this point later.

§ 5. CHANGES IN SALT SECRETION AS A RESULT OF ALTERATIONS OF THE OSMOTIC VALUE OF THE MEDIUM

When considering table 2 we can observe that the salt secretion appears to decrease every time the osmotic value of the medium is enhanced, even when the salt concentration is not changed. More salt proves to be given off if the osmotic value of the medium is lowered again.

The reduction was sometimes considerable. Thus the salt secretion was reduced to 75 % in experiment 4, when the osmotic value of a Hoagland solution was enhanced to 0.023 M (second period). When the osmotic value of the medium was still more increased (eighth period) a reduction to 35 % could be observed. The last value however, may be somewhat too small because of the periodical decrease of the salt secretion, since on a Hoagland solution also less salt seemed to be given off. However other experiments also show that the salt secretion is progressively reduced as the osmotic value of the medium is further enhanced. This can also be seen in the following experiment (experiment 16). The salt secretion was determined on various non-isotonic solutions of different salt concentrations. The increase of the salt concentration was now accompanied by an enhancement of the osmotic value of the medium. The salt secretion did not increase (compare experiment 2). However if we compare the amount of salt given off to the vessels while the plant was on a concentrated salt solution (2 H) with that given off under the same osmotic conditions but at a lower salt concentration (0.5 H + M) we see that the higher salt concentration still has some favourable effect.

Experiment 16:

	0.5 H (0.012 M)	2 H (0.030 M)	0.5 H (0.012 M)	0.5 H + M (0.031 M)	0.5 H (0.012 M)
S	0.83	0.76	0.89	0.61	0.87

It seems that there are two opposite effects: on the one side the salt secretion is stimulated by the higher salt concentration; on the other hand it is decreased by the higher osmotic pressure of the medium. This could mean that the uptake secretion is stimulated as the result of the higher salt concentration of the medium while the

tissue secretion is hampered; but it can also be imagined that both processes are hampered, so that the uptake secretion is not stimulated to the same extent as at lower osmotic values.

ARISZ, HELDER and VAN NIE also found an inhibition of the salt secretion at higher osmotic values of the outer solution. They supposed that more energy would perhaps be required to secrete salt into the vessels, as the sap would become more concentrated. If this were true an inhibition of the whole salt secretion should be expected.

Uptake and tissue secretion were determined in outer solutions of different osmotic values. To this end we determined how much salt was given off to the vessels when a plant was on a Hoagland solution, an isotonic solution of mannitol and a Hoagland solution again. This gave the value of the tissue secretion and the uptake secretion could also be calculated. The plant was then transferred to a Hoagland solution whose osmotic value had been enhanced by the addition of mannitol. After half an hour this solution was replaced by an isotonic solution of mannitol only, which was changed in its

TABLE 10

Changes in salt secretion resulting from changes of the osmotic value of the medium

Experiment	Osmotic value medium	Amount of salt given off on a Hoagland solution	Amount of salt given off on an isotonic mannitol solution	Difference (... uptake secretion)
16a.	0.014 M	0.70 ⁵	0.41	0.29 ⁵
	0.041 M	0.45 ⁵	0.23	0.25 ⁵
	0.014 M	0.71	0.42	0.29
16b.	0.014 M	1.56 ⁵	1.00	0.56 ⁵
	0.052 M	1.26	0.71	0.55

turn for the solution of salt and mannitol, again. At the end of the experiment the plant was put once more on an ordinary Hoagland solution to see whether there had been any changes in the rate of exudation or salt secretion as a result of periodicity. There were no such variations in experiments 16a and b, so the values found for uptake and tissue secretion may be considered reliable. It was now possible to calculate the values of uptake and tissue secretion when the outer solution had a higher osmotic value. The uptake secretion proved not to change when the osmotic value of the medium was enhanced [from 0.014 M to 0.052 M, as it amounted to 0.565 in the first case and to 0.55 in the latter. The tissue secretion however showed a decrease from 1.00 to 0.71 (table 10). It appears that in particular less salt is given off from the tissue when the osmotic value of the outer solution is higher. The transport to the vessels of salts taken up from the medium does not seem to be affected.

Although the osmotic value of the sap in this experiment increased to 0.067 M from 0.042 M on a Hoagland solution and 0.039 M on a solution of mannitol, this cannot be the cause of the decrease in the salt secretion.

It is possible to influence one part only of the salt secretion in this way. This supports our supposition that two different processes are involved.

The same phenomenon, of a decrease in the secretion of salts into the vessels as the result of higher osmotic values of the medium, is to be seen in LUNDEGÅRDH's tables (1945). Less potassium and nitrate was given off to the sap at higher glucose concentrations of the outer solution. LUNDEGÅRDH attributed this to a stimulation of the nitrate assimilation. However the same thing occurred when the concentration of potassium or calcium nitrate in the medium was very high, whereas smaller enhancements of the salt concentration stimulated the secretion of salts. This seems comparable to our experiment 16. We will discuss LUNDEGÅRDH's explanation later.

§ 6. THE INFLUENCE OF INHIBITORS ON THE EXUDATION

In preceding sections the hypothesis has been advanced that two different processes may be involved with the transfer of salts to the vessels. We have tried to gain a better insight into their mechanisms through influencing the salt secretion by means of inhibitors.

a. Potassium cyanide

It is known, that the exudation can be inhibited by the addition of KCN to the medium. According to VAN OVERBEEK (1942) a certain part of the exudation of tomato plants can be inhibited reversibly. ROSENE (1944) also stated that the exudation of *Allium* roots suffered an inhibition under the influence of KCN at higher concentrations (0.001 M). Lower concentrations could exercise a stimulating influence. ROSENE supposed that two processes would be involved, one of which was sensitive to KCN while the other was not; for the rate of exudation at first decreasing under the influence of the cyanide, after some time stayed at the same level. This hypothesis of ROSENE's does not necessarily imply an active and an inactive (that is osmotic) process, as VAN OVERBEEK thought. LUNDEGÅRDH (1949, 1950) found that the exudation of wheat plants was at first promoted; but that the rate of exudation decreased if the inhibitor was allowed to act for a longer time.

Experiment 17 (fig. 13). It was our intention to investigate the influence of KCN on the tissue exudation as well as on the part of the exudation depending on the presence of salt in the medium. The exudation was first examined under normal conditions: rate of exudation and salt secretion were compared when a plant was on a Hoagland solution and on an isotonic solution of mannitol. When, on a Hoagland solution again, an equilibrium had been attained KCN (0.0005 M) was added. This caused the rate of exudation to decrease immediately. The rate of decrease slowed down and after 25 minutes the rate of exudation was almost steady. At that time a new solution, again a Hoagland solution with 0.0005 M KCN, was added, for the concentration of KCN can diminish rapidly in a non-

alkaline solution that is well aerated. This might be the reason why the rate of decrease was slowing down. When the outer solution had been renewed the rate of exudation did indeed decrease somewhat further.

After the plant had been on a solution of KCN for one hour, an investigation was made to see whether a salt effect could still occur: The Hoagland solution was replaced by a solution of mannitol con-

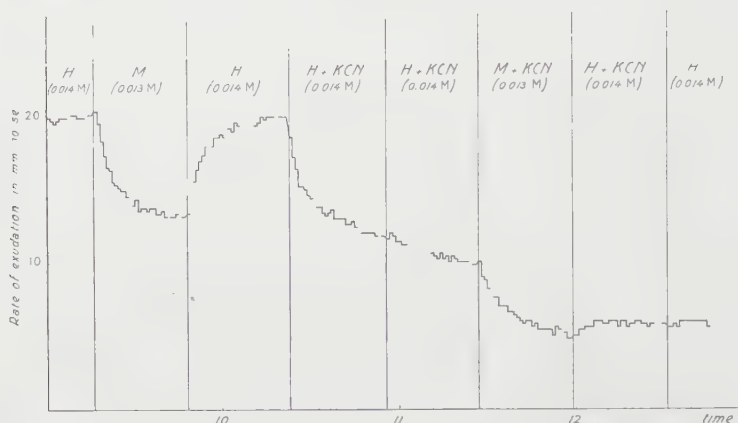


Fig. 13. Changes in the rate of exudation caused by the addition of 5.10^{-4} M.KCN to the outer solution. Comparison of the salt effect under normal conditions and in the presence of KCN.

taining as much KCN and the resulting decrease in the rate of exudation indicated that the uptake secretion was still active; but when, half an hour later, a Hoagland solution (with KCN) was given once again, the rate of exudation showed hardly any increase.

The rate of exudation proves to decrease under the influence of KCN. The course of this decrease bears a remarkable resemblance to that caused by the removal of salt from the medium. One might be inclined to suppose that an inhibition of the uptake secretion would be involved here. However, the situation seems to be less simple than this. In fig. 13 we can see how the rate of exudation is diminished to 12 mm/30 sec when KCN is added to a Hoagland solution. The rate of exudation amounted to 13 mm/30 sec when no salts were present in the medium (second period). This indicates that tissue exudation must have been partially inhibited at any rate, even if the exudation depending on the uptake of salts had been inhibited completely. The latter supposition proved however to be false, since the removal of salts induced a further decrease of the rate of exudation (sixth period). We must however point out that the decrease caused by the replacement of a salt solution by a solution without salts appears greater than it really is. The rate of exudation was still decreasing at the moment when the salts were removed (sixth and seventh period). The big decrease then observed must be attributed to the change of the medium, but the value attained in the condition

of equilibrium was controlled by two factors: the decrease in the rate of exudation resulting from the lack of salts, and the prolonged inhibition under the influence of KCN. This is indicated by the fact that the rate of exudation did not increase very much when, afterwards, salts were added again (eighth period.) The osmotic value of the sap decreased when the salt concentration of the medium was diminished, but otherwise it did not show any changes. It appears that when KCN is added to the medium less salt is given off to the vessels; because the osmotic value of the sap did not change we must conclude that the reduction of the salt secretion may not be the only cause of the decrease in the exudation, since if in the formula $b = k (\bar{O}_b - \bar{O}_m)$, b decreases while $\bar{O}_b - \bar{O}_m$ is not changed, k must decrease too. In this experiment k was not determined. Fuller reference to this point will be made later.

The exudation was only partially inhibited in this experiment. After approximately one hour and a half the exudation did not seem to decrease any further, although we must reckon with the possibility that the concentration of KCN was in the meantime reduced. It appeared to be possible to inhibit the exudation completely by using higher concentrations (0.001 M) but this took several hours although a decrease set in as soon as KCN had been added to the outer solution.

A stimulation of the exudation was never observed with the concentrations used (0.0001–0.005 M). Big differences appear to exist between the sensitivity of individual plants to KCN. The material used in 1951, that had been grown in an ordinary green-house, was on the whole less sensitive than the plants which were used later and which had been cultivated in the artificially lit green-house. In the first case a concentration of 0.005 M KCN might not cause a complete inhibition, the inhibition being furthermore fairly well reversible. When the more sensitive plants were used the exudation recovered partially or not at all when KCN was removed from the medium. It may however be observed, that BROUWER (1953) found that it took sixteen hours before intact plants of *Vicia Faba* recovered from treatment with KCN. Our experiments were not continued so long.

It has been already mentioned that experiment 17 indicates not only that less salt is given off to the vessels, but also that k is changed under the influence of KCN. This was investigated in more detail in the following way: the changes in the rate of exudation were not followed continuously, but the average rate of exudation during five minutes was determined every half hour. A sample of sap was taken at the same time to determine its osmotic value. Then the plant was put on a more concentrated solution for a few minutes so that k could be calculated from the sudden decrease in the rate of exudation. The data of such an experiment are given in table 11. The rate of exudation decreased, if KCN was added to the medium, but the osmotic value of the sap remained constant, the salt secretion therefore changing to the same extent as the rate of exudation. K also showed a decrease. Conversely the increase in the rate of exudation after the removal of KCN, was accompanied by an increase of the salt secretion as well as of k .

The exudation of *Sanchezia nobilis* also decreased on the addition of KCN. The course of this change was identical to that described in experiment 17: the decrease was rapid at first, but afterwards slowed down without altogether ceasing during the experiment. Tissue as well as uptake secretion was inhibited although a distinct salt effect could occur still one and a half hour after KCN had been added.

TABLE 11

Effect of KCN and DNP on rate of exudation, salt secretion and conductivity for water

Exp.	Medium	time	<i>b</i>	O_b	<i>S</i>	<i>k</i>
17a	Mannitol	10.30	11.8	0.023	0.27	840
	" " " " " " " " " "	11.00	11.6	0.022	0.26	800
	0.005 M KCN	11.30	7.6	0.019	0.14	540
	" " " " " " " " " "	12.00	7.0	0.020	0.14	560
	Mannitol " " " " " " " " " "	12.30	9.0	0.020	0.18	620
	" " " " " " " " " "	13.00	8.6	0.022	0.19	660
	0.005 M KCN	13.30	6.4	0.021	0.13	360
	distilled water	10.15	6.5	0.020	0.13	241
18a	" " " " " " " " " "	10.45	7.5	0.024	0.18	289
	10 ⁻⁴ M DNP	11.15	6.0	0.022	0.13	241
	" " " " " " " " " "	11.45	4.5	0.021	0.09	144
	distilled " water	12.15	4.7	0.020	0.09	151
	" " " " " " " " " "	12.45	5.1	0.020	0.10	187
	" " " " " " " " " "	13.15	6.4	0.020	0.12	244
	" " " " " " " " " "					

The osmotic value of the sap did not change, so that here *k* must have decreased too. Both exudation and salt secretion proved to recover fairly well, when the solution of KCN was removed.

So we have found that KCN can inhibit the exudation. The inhibition proved in some cases to be complete so that there is no reason to suppose that a certain part of the exudation process is not affected, although we found that after some time the rate of exudation did not decrease further when weak concentrations were used. The course of the decrease was very much like that caused by an inhibition of the salt secretion resulting from the removal of salts from the medium. The decrease however, cannot be explained in a similar way, its causes are complicated. It has been pointed out already that the tissue secretion is inhibited besides the uptake secretion. This is clearly demonstrated when KCN is added to a solution without salts: the rate of exudation then decreased in exactly the same way as when in fig. 13, KCN was added to a Hoagland solution (fourth period). The whole process of salt secretion must be concerned here. We cannot say, if this decrease in the salt secretion takes place immediately KCN is added, because the decrease then observed is caused not only by a reduction of the salt secretion and the consequent decrease in the osmotic value of the sap, but also by a decrease of the water conductivity of the roots, the factor *k*.

Although both uptake and tissue secretion were inhibited, it is shown by fig. 13 that the first is inhibited to a greater extent. The salt effect had become very small two hours after KCN had been

added. While the rate of exudation on a solution without salts had decreased from 13.2 mm/30 sec to 5 mm/30 sec, the tissue secretion itself had decreased in that time from 0.52 to 0.21. This perhaps is not so surprising since it is to be expected that the uptake of salts will be completely inhibited by a similar concentration of KCN. MACHLIS (1944) for instance found, that the uptake of bromide by barley roots was fully inhibited after a treatment of two hours with KCN. 0.0001 M. KCN would according to LUNDEGÅRDH entirely inhibit the anion respiration of wheat roots which controls the uptake of anions. Again WEEKS and ROBERTSON (1950) found with carrot tissue an inhibition of salt uptake by KCN.

b. 2,4-Dinitrophenol

The qualitative action of 2,4-dinitrophenol did not prove to differ much from that of KCN. Like KCN it caused a decrease in the rate of exudation that set in immediately, both when the outer solution contained salts as when it did not. The osmotic value of the sap was not changed. It follows from this, that the salt secretion changed to the same extent as the rate of exudation. The tissue secretion must certainly be inhibited as DNP affected the exudation when no salts were present in the medium. The influence of DNP on the part of the exudation that depends on the uptake of salts was investigated in the same way as in experiment 17: with the same plant the salt effect was investigated under normal conditions and in the presence of DNP. Fig. 14 shows that both uptake and tissue secretion were diminished. Rate of exudation and salt secretion decreased to approximately one half during the experiment. After the inhibitor had been removed from the medium, the rate of exudation increased gradually; but after two hours it had not yet completely recovered.

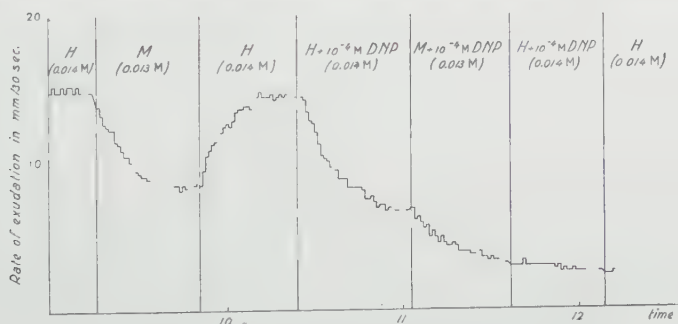


Fig. 14. Influence of dinitrophenol on the salt effect.

It is difficult to say to what extent the exudation is influenced in these experiments by the periodicity. The fact that the rate of exudation was very regular at the beginning — before addition of the inhibitor — indicates that periodicity is not very important here. Periodical changes were rather small with plants grown in the artificially illuminated green-house as will be demonstrated in section 7.

In these experiments such plants were chiefly used, so it is probable that the decrease in the rate of exudation, salt secretion and k , is for the greater part at least caused by the inhibitor, although the possibility of small periodical changes especially at the end of the experiment, cannot be ruled out altogether.

The sensitivity of plants to DNP proved to be rather variable. The same concentration that was used in experiment 18 (fig. 14) caused in other cases an inhibition of 15–20 % only, which was completely reversible.

It has been pointed out already that the osmotic value of the sap did not change under the influence of dinitrophenol. A decrease of k should therefore be expected. Table 11 (exp. 18a) shows that this was actually the case.

It is known that DNP uncouples respiration and phosphorylation processes. The uptake of salts by carrot tissue is inhibited by DNP (ROBERTSON, WILKINS and WEEKS, 1951), but as we explained while discussing the action of KCN, a decrease of the salt uptake cannot be the only cause of the decrease in the exudation. ARISZ (1953) found that the uptake of phosphate and asparagine by tentacles of *Drosera* was inhibited. ROBERTSON, WILKINS and WEEKS mentioned the possibility of an inhibition of the transport of salts by DNP. This would explain the simultaneous inhibition of uptake and tissue secretion. It should be clearly understood however, that the experiments of ROBERTSON, WILKINS and WEEKS concern the transport of salts to the vacuole and our experiments the transport to the vessels.

ARISZ (1953) did not find any influence of DNP on the transport of chloride through the cytoplasm of *Vallisneria*, but the secretion of salt into the vacuole proved to be inhibited.

c. Sodium arsenate

Experiment 19 (fig. 15). Addition of 10^{-5} M sodium arsenate to the medium (distilled water) caused a decrease of the rate of exudation. This decrease set in immediately and passed off in the same way that has been described in the experiments with KCN and DNP. The osmotic value of the sap did not change, so in this case too less salt must have been given off — from the tissue —, and the water conductivity of the roots must have decreased. The exudation did not recover when the solution of Na-arsenate was replaced after half an hour by distilled water. From distilled water the plant was transferred to a Hoagland solution. After 40 minutes this solution was changed for distilled water again. Now the rate of exudation proved to have increased to its initial value. Addition of Na-arsenate — a solution ten times more concentrated this time — caused the exudation to decrease once again.

The inhibition caused by Na-arsenate appears to be reversible, but it is remarkable that the exudation recovered only after the plant had stayed on a salt solution for some time.

WARBURG and CHRISTIAN (1939) found in experiments on isolated enzyme systems that arsenate can replace inorganic phosphate in

oxidative reactions, for which the uptake of inorganic phosphate is necessary. BONNER (1950) stated that the growth of *Avena coleoptiles* induced by indole acetic acid was inhibited by Na-arsenate, but that this inhibition could be overcome by adding phosphate to the medium. In the light of these data it seemed probable that the reversal of the arsenate inhibition described in experiment 19 would be the result of the presence of phosphate in the Hoagland solution. It has indeed been proved that exudation and salt secretion did not recover on a

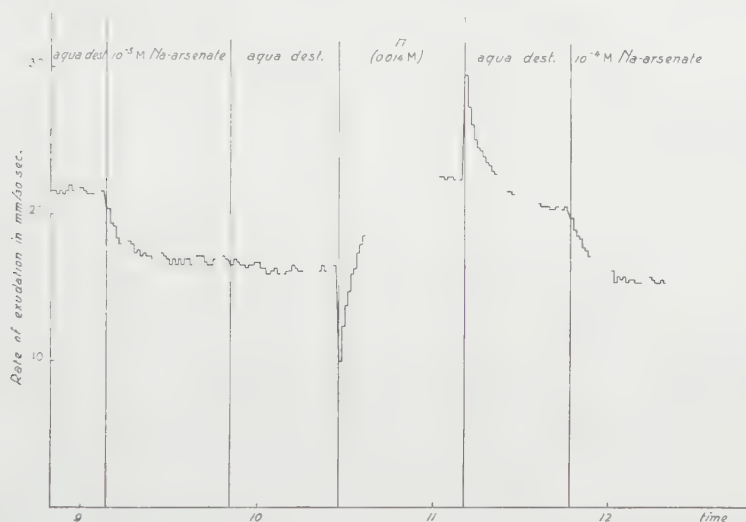


Fig. 15. Reversible decrease of the rate of exudation caused by adding sodium arsenate.

solution containing all the salts from a Hoagland solution with the exception of phosphate. On the other hand no decrease of the exudation could be observed if arsenate was added to a complete Hoagland solution; if a Hoagland solution without phosphate was used the exudation decreased (the absence of phosphate by itself is unimportant c.f. section 3a). For this reason a Hoagland solution without phosphate was used in the experiments designed to investigate the influence of arsenate on exudation.

It has been shown in experiment 19 that the tissue secretion decreased on the addition of Na-arsenate to the outer solution. The influence of Na-arsenate on the part of the exudation governed by the uptake of salts was investigated in the way described before. In fig. 16 it is demonstrated that the rate of exudation decreased from 19.4 mm/30 sec to 13.6 mm/30 sec when a Hoagland solution was changed for a solution of mannitol. The addition of 10^{-4} M Na-arsenate caused a very small decrease, that could be completely reversed, but 10^{-3} M arsenate made the rate of exudation decrease from 18.4 to 14.4 mm/30 sec and the rate of exudation showed a further decrease to 10.6 mm/30 sec if the Hoagland solution was now

replaced by a mannitol solution (also containing arsenate). The osmotic value of the sap changed only when the salt concentration of the medium was lowered. Thus the osmotic value decreased from 0.042 M to 0.038 M in the second period and from 0.042 M to 0.036 M, when the Hoagland solution was replaced by the mannitol solution in the presence of arsenate. This means that both tissue and

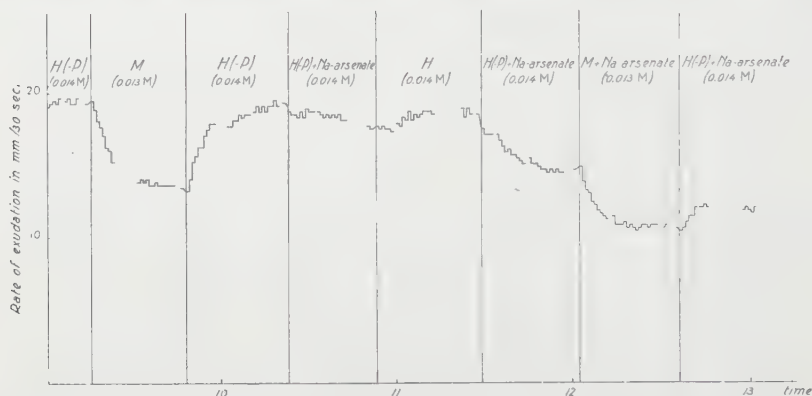


Fig. 16. Influence of sodium arsenate on the rate of exudation in the presence and absence of salts. In the fourth period 10^{-4} M sodium arsenate was added, in the sixth and following periods 10^{-3} M.

uptake secretion are diminished. This conclusion could have been drawn already from the fact that the removal of salts caused a smaller decrease of the exudation if arsenate was present, but in that case we must reckon with the possibility that k has become smaller. The same decrease of the salt secretion could then cause a smaller decrease in the rate of exudation.

In addition to the changes of rate of exudation and salt secretion a decrease of k has been demonstrated.

d. Sodium fluoride

LUNDEGÅRDH (1949, 1950) found that the exudation of young wheat plants was inhibited by NaF in concentrations of 0.01–0.001 M. The osmotic pressure of these solutions was considered unimportant. LUNDEGÅRDH first concluded that the sensitivity of the exudation to NaF would depend on an exudation of cations to the vessels. This process would be controlled by glycolysis. Later experiments (1950) lead to another conclusion, since it appeared that the rate of exudation, that is the amount of water given off to the vessels, did indeed decrease under the influence of NaF, but the secretion of nitrate did not. The nitrate concentration of the sap even proved to show a great increase. LUNDEGÅRDH now supposed that the exudation of salts was not linked to anaerobic glycolysis but that it was independent of metabolism. The decrease in the exudation of sap would be the result of an inhibition of the so-called "extra-water" secretion,

the exudation of water released in metabolic processes. This extra water could in his opinion amount to $2/3$ – $3/4$ of the total sap.

LUNDEGÅRDH's experiments were repeated with tomato plants.

Most experiments were carried out with solutions containing 0.005 and 0.01 M NaF, at a pH of 5.2. The trouble about using such high concentrations in our experiments is that the solutions impede the exudation on account of their osmotic value. This is shown in table 12. The rate of exudation decreased when 0.01 M NaF was added to a salt solution, while the osmotic value of the sap rose from 0.036 M to 0.043 M. When however the salt solution was replaced by a solution of salts and mannitol isotonic with the solution of fluoride, the result was the same. The course of the change in the rate of exudation on the addition of NaF (a sudden fall followed by a gradual increase) also demonstrated that an osmotic effect was concerned. So it proved to be necessary to make the solutions without NaF isotonic with those which contained it by adding mannitol. It was impossible to use a Hoagland solution as fluoride precipitated with the Ca present. Instead of an ordinary Hoagland solution one was used in which $\text{Ca NO}_3 \cdot 2$ had been replaced by KNO_3 or a solution containing 0.005 M KNO_3 and 0.0005 M KH_2PO_4 . It has been demonstrated that the absence of Ca ions does not affect the exudation. Lower concentrations than 0.005 M NaF did not affect the exudation at all, even when they were allowed to act for several hours. Sometimes even when greater amounts of NaF were added no effect could be

TABLE 12

Changes in rate of exudation and osmotic value of the exudation sap caused by the addition of 0.01 M NaF, compared with changes resulting from an enhancement of the osmotic value of the medium (experiment 21).

Medium	time	<i>b</i>	O_b	S
0.005 M KNO_3 (0.013 M) . . .	10.40	9.6	0.036	0.35
0.005 M KNO_3 + M (0.037 M) . . .	11.05	6.0	0.044	0.28
0.005 M KNO_3 + M (0.037 M) . . .	11.30	7.6	0.042	0.32
0.005 M (0.013 M) . . .	12.00	11.4	0.036	0.41
0.005 M + NaF (0.036 M) . . .	12.30	9.0	0.043	0.40
0.005 M + NaF (0.036 M) . . .	13.20	7.4	0.044	0.33
0.005 M + NaF (0.036 M) . . .	13.50	7.4	0.043	0.33
0.005 M (0.013 M) . . .	14.20	10.2	0.037	0.39
0.005 M + M (0.037 M) . . .	14.50	6.0	0.044	0.37

observed. In other experiments the rate of exudation decreased at the addition of NaF. This decrease set in immediately but it was usually rather slight.

The pH of the medium was shown to be very important for the action of NaF. Thus the rate of exudation decreased in an hour to one half, if 0.01 M was added at a pH of 4.3; the same concentration causing no decrease at all at pH 5.2. The change of pH itself did not affect the exudation (c.f. section 3c).

Thus the rate of exudation could be inhibited by NaF. The osmotic value of the sap, however, did not change. This means that less salt was given off to the vessels. An inhibition of the whole salt secretion

proved to be concerned: both uptake and tissue secretion were decreased. We could not confirm LUNDEGÅRDH's results, as a decrease of the amount of water given off to the vessels and a simultaneous increase of the osmotic value of the sap were found to result only from the osmotic action of the fluoride solution. It must be pointed out, however, that LUNDEGÅRDH's hypothesis is not the only possible explanation of his results. Similar phenomena would be observed if the salt secretion was not affected but only the water conductivity of the roots (compare the influence of the pH of the outer solution on the exudation). It appears from LUNDEGÅRDH's figures that the inhibition caused by NaF increased with time. This would not happen if the action of NaF was osmotic only for then an equilibrium would be attained after a brief time; but it might be the result of a gradual decrease of k .

The effect of NaF was proved to differ little from that of the other inhibitors mentioned earlier. The only remarkable thing was the great influence of the pH of the medium. LUNDEGÅRDH also found a greater effect at a lower pH, but in his experiments the exudation was already considerably inhibited at pH 5.

e. Iodo acetic acid

The inhibitors discussed up to now appeared to affect both the uptake and tissue exudation. It was to be expected that iodo acetic acid would influence the uptake secretion at least, since it is known that the uptake of salts can be inhibited by this substance. MACHLIS (1944) demonstrated that barley roots did not take up salt if JA was present. LUNDEGÅRDH (1949) found that 0.0002–0.001 M JA at a low pH inhibited the uptake of chloride and nitrate almost completely and decreased the rate of exudation.

Low concentrations such as 0.0001 M were proved not to affect the exudation of tomato plants, at least at a pH of approximately 5. A solution ten times more concentrated influenced the exudation at first no more than the weaker concentration; but after 20–30 minutes the rate of exudation began to decrease gradually. The decrease in the exudation, however, followed a somewhat different course to that observed with the other inhibitors. In their case a decrease was always observed that was rapid at first and slower afterwards (fig. 13, 14) but the rate of exudation now decreased continuously at the same rate so long as the inhibitor was present in the outer solution. The decrease started sooner when the concentration of the inhibitor was higher.

The osmotic value of the sap decreased only when the rate of exudation had decreased considerably; otherwise it remained unaltered. As in the preceding experiments a simultaneous decrease of salt secretion and k would be expected, but in some cases the first seems to decrease to a greater extent.

It appears from fig. 17 that both uptake and tissue secretion were inhibited, but the removal of salts from the medium caused a still further decrease of the exudation even in the presence of JA. This

indicates that the uptake of salt was not fully inhibited. It is difficult to determine to what extent the part of the exudation that depends on the salt uptake had been inhibited, as the decrease of the exudation caused by the lack of salts was enhanced by the prolonged action of JA, in the way that has been described in section 6a. After $4\frac{1}{2}$ hours

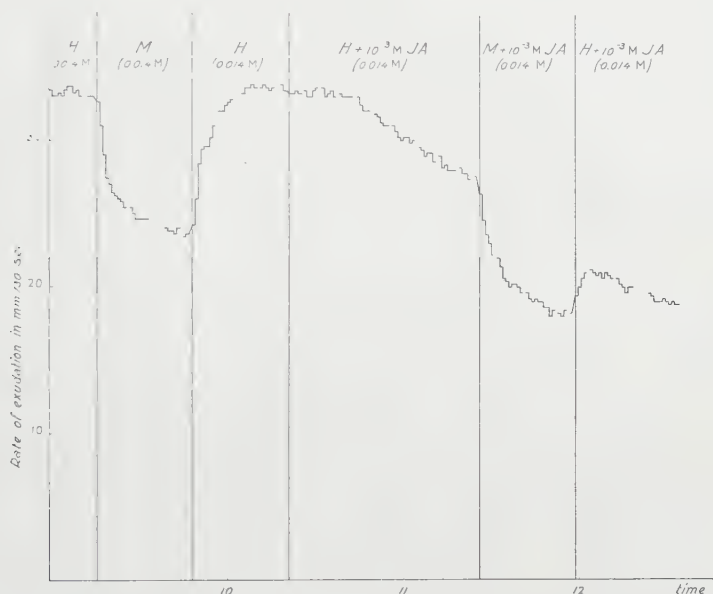


Fig. 17. Inhibition of the rate of exudation under the influence of iodo acetic acid.

no further salt effect occurred. The uptake secretion seems to be completely inhibited at that time, while the tissue secretion is, as yet, not. The rate of exudation did not increase when a solution of JA was replaced by a solution without this substance, but it did not decrease further either. It has been shown by BONNER and THIMANN (1950) that the iodo acetic acid inhibition of the growth of *Avena* coleoptiles could be overcome by addition of a dicarboxylic acid, malic acid for instance. The inhibition of the exudation, however, did not seem to be affected by the addition of malic acid. No change of exudation occurred if 0.01 M malic acid was added to the outer solution (the pH of the medium had been kept the same, by adding KOH). Neither exudation nor salt secretion increased even when the roots had been on a solution of malic acid for several hours.

f. Phenylurethane

We have up to now described the influence of several substances which are known to act on certain enzymatic processes. Following is an experiment in which the effect of a narcotic, phenylurethane, was investigated. SPEIDEL's experiments (1939) had demonstrated that this substance is able to diminish the exudation of several plants

while HEYL (1930) found that narcotics could affect the exudation of *Sanchezia*.

With tomato plants the rate of exudation appeared to decrease as soon as phenylurethane (0.001 M) was added to the medium (fig. 18). This decrease followed the same course as in the experiments with KCN, DNP, Na-arsenate and NaF. The decrease in the rate of exudation was accompanied by a decrease of the salt secretion. The exudation on a solution lacking salts proved to diminish after the addition of phenylurethane. As furthermore the removal of salts from the medium resulted in only a small reduction of the exudation when phenylurethane was present, we must conclude that both parts of the exudation had been decreased. The action of phenylurethane corresponded in this respect with that of the other inhibiting substances.

The inhibition proved to be more or less reversible: exudation and

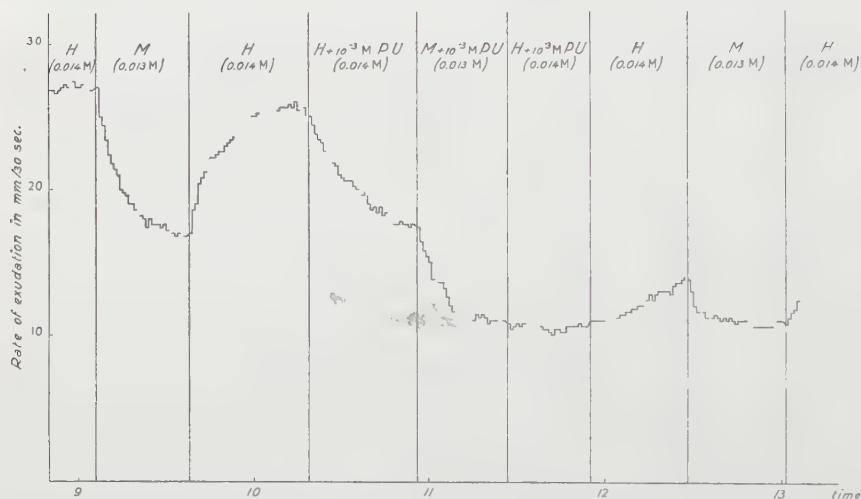


Fig. 18. Influence of phenylurethane on the rate of exudation, in the presence and absence of salts.

salt secretion showed a gradual increase when the solution of phenylurethane was removed. It is remarkable that the recovery of the exudation after removal of phenylurethane seems in the first place to be due to a recovery of the uptake secretion. It appeared that when the salt solution was changed for a solution of mannitol (seventh and eighth period) the rate of exudation decreased to the same level as before in the presence of phenylurethane (fifth and sixth period). This means that the tissue secretion had not yet increased at that time, while the total salt secretion had. The experiment has not been continued, but there is no reason to suppose that it is impossible that the tissue secretion might also increase after a longer time.

Recapitulating we can state that the rate of exudation decreased as the result of adding potassium cyanide, 2,4-dinitrophenol, sodium arsenate, sodium fluoride, iodo acetic acid or phenylurethane to the

medium. Less salt was given off to the vessels, and the water conductivity of the roots decreased; that is to say both water transport and salt transport were affected. The whole salt secretion decreased, but in some cases the transport of salts taken up from the medium was the first to be completely inhibited.

It is remarkable that the exudation reacts so rapidly to the addition of inhibitors (except iodo acetic acid). LUNDEGÅRDH (1949, 1950) also found a rapid effect of KCN: the exudation was already considerably decreased ten minutes after KCN had been added to the medium. He found in his investigation of the inhibition of the cytochrome cytochromeoxidase system by KCN (1952) that the cytochromeoxidase was considerably inhibited in 30 seconds. He concluded on the strength of these results that the cytochromeoxidase is localized at the cell surface. Other substances besides KCN have, however, been proved to act as rapidly, although they are supposed to affect different enzymatic processes. So we must suppose either that all these substances can penetrate the roots very rapidly, or that an important part of the exudation process is localized near the root surface.

The pH of the medium proved to be very important to the action of NaF. The effect of other substances has been investigated at one pH value only, but it is probable that their effect will also be influenced by the pH (c.f. LUNDEGÅRDH 1949). The pH of the medium affects the relation between undissociated molecules and ions, which is known to be important for the action of such substances. The effect is greater as the concentration of undissociated molecules is higher (SIMON and BEEVERS 1952).

From these experiments we can draw only a few conclusions about the way in which these substances affect the exudation process. As the modes of action of various inhibitors are so much alike (JA excepted) it seems plausible to suppose that no specific inhibition of the exudation is concerned, but that the action is more indirect; that is to say that metabolic processes might be affected, which are important for the exudation process. The fact that a narcotic exercises the same influence as substances acting on several enzymatic reactions, also points this way. HOAGLAND and BROYER (1942) stated that the metabolism of decapitated tomato plants was affected by KCN in a similar way as by oxygen deficiency. In both cases less bromide was taken up and the permeability of the tissue decreased. STENLID (1949) thought it possible that the structure of the protoplasm would be changed under the influence of DNP. Such an effect might explain the simultaneous decrease of the transport of salts and water.

In consequence of LUNDEGÅRDH's experiments (1949) in which the exudation was stated to be inhibited by high concentrations and stimulated by lower concentrations of indole acetic acid, the influence of this substance on the exudation of tomato plants was investigated. It seemed on account of the investigations of VON GUTTENBERG and BEYTHIEN (1951) and BRAUNER and HASMAN (1952) that in particular changes of the conductivity for water should be expected although

we must bear in mind that in these experiments the water permeability of tissue was investigated and not that of intact roots. SKOOG, BROYER and GROSSENBACHER (1938) found a stimulation of the exudation of *Helianthus*, if indole acetic acid was applied to the stem. More salt was given off to the vessels in this case.

Like LUNDEGÅRDH we added indole acetic acid to the outer solution. The influence of indole acetic acid on the exudation proved on the whole to be very small. A stimulation could not be ascertained with sufficient certainty. Higher concentrations (1–10 mg/L) caused a decrease in the rate of exudation, but k was hardly changed. The osmotic value of the sap decreased somewhat so that the decrease in the rate of exudation must be attributed chiefly to a decrease of the salt secretion. This inhibition occurred later than in the experiments with inhibitors described earlier, and was not so strong.

§ 7. PERIODICITY

Authors like HOFMEISTER (1862) and WIELER (1893) have already demonstrated that the rate of exudation of various plants shows fluctuations during the day, so that the greatest amount of sap is given off approximately at noon while a minimum occurs in the night. HEYL (1930) attributed this phenomenon to fluctuations of temperature during the experiments. However, it was also observed by others when the temperature was kept constant (HOFMEISTER; SPERLICH and HAMPEL, 1936). GROSSENBACHER (1939) stated that, with *Helianthus*, periodicity could be influenced by several circumstances: The exudation of plants grown under normal conditions, showed a maximum by day and a minimum during the night, whereas the reverse happened when the plants had been kept in darkness during the day and had been illuminated overnight. This seemed to indicate that the periodicity of exudation was caused by the alternation of periods of light and darkness during growth, as SPERLICH and HAMPEL thought. However, periodicity also occurred, with plants that had been grown in constant light, or in permanent darkness (that is in weak red light); the moment at which maxima and minima occurred now depending on the time of decapitation. Changes of temperature could also exercise some influence.

SPEIDEL (1939) also found a relation between the moment at which the plants had been decapitated and the time at which the maxima and minima were observed, when investigating the exudation of several plants (*Helianthus*, *Ricinus* and others). The nature of the medium was also of importance: plants grown in sand showed their first maximum approximately 12 hours after decapitation (this period seems rather variable), plants in a nutrient solution 12 hours later. The maxima could be shifted by a rise in temperature. HEIMANN (1952) came to a similar conclusion: the time of decapitation was of importance for the time at which the maximal exudation could be observed with *Kalanchoe Blossfeldiana*. The rhythm could be influenced by illumination; light having a stronger influence as more leaves were left on the exuding stump.

According to WHITE (1938) the phenomenon of periodicity should be independent of external conditions, as isolated tomato roots, that had been cultivated for five years, showed a diurnal fluctuation of root pressure. Changes of temperature were considered unimportant, while the influence of light might in this case be left out of consideration.

A diurnal periodicity has been demonstrated not only in exudation but also in guttation. The cycles of guttation could be influenced by changes in the periods of illumination (ENGEL and FRIEDRICHSEN, 1951, 1952; HEIMANN 1950). It appears from this, that such a rhythm also occurs in intact plants so that it cannot be considered a result of decapitation (c.f. SPEIDEL).

Changes of permeability have repeatedly been supposed to be involved with the periodicity of exudation (GROSSENBACHER 1938; SKOOG, BROYER and GROSSENBACHER 1938; ENGEL and FRIEDRICHSEN 1952), but it seems that no investigation has ever been made to find out whether the permeability does actually change. SKOOG, BROYER and GROSSENBACHER found that the amounts of salt present in the sap of *Helianthus annuus* also fluctuated, while the periodicity could be intensified by applying indole acetic acid to the stem.

When describing the experiments with tomato plants we pointed out repeatedly that irregularities in the exudation might be the result of periodic changes in the rate of exudation. The observation of such irregularities was, in connection with the data from the literature just mentioned, a reason for investigating the course of the exudation rate, salt secretion and k over several successive days. In table 13 the results are given of an experiment that has been carried out in the following manner: rate of exudation, osmotic value of the sap and k were determined every hour in precisely the same way that has been described in section 6 (experiment 17a). The nutrient solution was renewed every two hours in these experiments to avoid important changes of the salt concentration of the medium.

We see in experiment 24 that the rate of exudation was 9.9 mm/30 sec at 7 a.m.; it increased to 21.1 at 11 and afterwards decreased to 7.7 mm/30 sec at 7 p.m. On the second day the rate of exudation was much slower than on the first, but it proved to have increased when compared with the last observation of the previous night. During the night no observations were made, but the sap was collected during that time, so that the average rate of exudation could be calculated. This amounted to 3.2 mm/30 sec, which was less than both the last value observed in the evening and the first of the next morning. This indicates that still a lower value must have been attained during the night. So the exudation of tomato plants was actually proved to show a maximum by day and a minimum at night. The second day a maximum was found once more between 11 and 14 hours; this cannot be seen in experiment 24 as the circumstances were changed at 11 o'clock.

The osmotic value of the sap proved to change very little during the day. A small decrease occurred by the end of the afternoon. No further important changes could be observed the second day. It

TABLE 13
Periodic changes of rate of exudation and conductivity for water under various conditions.
* means that sucrose has been added to the medium

time	24 Tomato Hoagland solution			24a Tomato distilled water			24b Tomato Hoagland solution containing 0.02 M sucrose			24c Tomato Hoagland solution leaves in sucrose			24d Tomato Hoagland solution, Decapitated at 17.30 8.30			24e Sanchezia nobilis. Hoagland solution		
	b	O _b	k	b	O _b	k	b	O _b	k	b	O _b	k	b	O _b	k	b	O _b	k
7	9.9	0.032	387	—	—	—	22.9	0.040	950	10.0	0.048	258	—	—	—	—	—	—
8	14	0.032	568	11.1	—	422	29.7	0.044	1119	13.0	0.047	329	11.0	—	—	—	—	—
9	—	0.032	615	11.0	0.026	403	32.7	0.050	945	15.5	0.046	396	12.0	2.2	—	—	—	—
10	16.4	0.030	694	16.3	0.022	553	32.7	0.047	906	17.9	0.049	454	12.4	3.5	—	—	—	—
11	20.5	0.031	742	14.7	0.022	561	32.9	0.047	906	17.9	0.049	454	12.6	4.4	—	—	—	—
12	27.1	0.031	658	12.2	0.018	554	34.6	0.044	869	19.0	0.045	471	12.6	4.4	—	0.028	706	—
13	17.0	0.031	584	11.0	0.018	483	31.3	0.045	825	18.7	0.041	446	12.9	5.0	—	0.028	740	—
14	14.5	0.029	580	11.3	0.016	411	32.6	0.051	800	16.1	0.043	379	12.0	5.6	—	0.027	765	—
15	13.5	0.029	516	8.7	0.016	350	31.3	0.045	806	14.0	0.044	358	12.0	6.3	—	0.028	669	—
16	12.5	0.032	503	8.5	0.018	353	29.0	0.047	737	11.9	0.043	318	11.4	6.3	—	—	—	—
17	11.1	0.029	484	6.8	0.019	355	21.9	0.045	604	9.8	0.044	296	11.8	6.6	—	—	—	—
18	9.0	0.027	436	5.0	0.017	280	18.5	0.051	500	8.1	0.045	238	11.0	6.6	—	—	—	—
19	7.7	0.026	336	3.6	0.015	217	12.2	0.045	313	5.1	0.043	175	—	—	—	—	—	—
20	6.0	0.025	274	2.4	0.018	148	9.6	0.044	252	4.0	0.042	139	—	—	—	—	—	—
21	4.9	0.026	242	2.6	0.015	—	—	—	—	—	—	—	—	—	—	—	—	—
22	4.2	0.027	219	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22-7	3.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	3.9	0.022	213	—	0.011	—	3.7	0.046	148	3.0	0.039	100	—	—	—	24.5	0.029	761
8	3.9	0.021	200	3.4	0.010	479	4.0	0.048	154	3.9	0.022	100	—	—	—	24.9	0.029	725
9	4.4	0.019	226	4.4	0.011	513	4.5	0.046	185	4.5	0.039	117	15.2	15.7	—	25.4	0.026	692
10	5.0	0.022	232	5.6	0.013	547	5.3	0.044	177	6.3	0.041	127	17.0	16.6	—	25.4	—	708
11	6.0	0.024	245	7.5	0.013	563	5.5	0.045	138	6.7	0.040	137	18.4	17.7	—	24.4	0.031	704
12	8.5*	0.024*	254*	8.8	0.011	547	5.5	0.044	123	5.9	0.040	118	19.2	19.3	—	24.2	0.030	704
13	15.0*	0.031*	250*	—	—	—	—	—	—	—	—	—	18.8	19.5	—	23.8	0.032	700
14	11.5*	0.033*	209*	9.8	0.013	528	—	—	—	—	—	—	18.8	18.8	—	20.2	0.033	554
15	—	—	—	—	—	—	—	—	—	—	—	—	16.6	17	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	15.2	16.2	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	12.6	14.2	—	—	—	—

follows from this first that a maximal quantity of salt was given off at the time when the rate of exudation was largest (table 13) and secondly that k must have changed too. Indeed k proved to increase from 387 at 7 a.m. to 658 at 11 a.m. and to decrease afterwards. At 7 a.m. the value of k amounted to 336.

This experiment was carried out with a plant that had been grown under normal conditions of illumination. Corresponding results were obtained with all such plants, although individual differences might occur, so that the changes were sometimes less distinct. In the same table data are recorded of an experiment that had been conducted in the same way; the medium however, consisted not of a Hoagland solution but of distilled water. Exudation, salt secretion and k showed a maximum between 10 and 12 a.m., just as in the former experiment. The changes in magnitude of these three ran parallel as in the other case. The second day however, the rate of exudation had much decreased and less salt was given off, but k was as large as on the previous morning. This proved to be a typical difference between plants on a salt solution and those on distilled water.

Thus we have seen that the periodic changes in the rate of exudation of tomato plants are accompanied by changes in the amount of salts given off to the vessels and in the conductivity for water of the roots. The salt secretion appeared to have greatly decreased one day after decapitation. Depletion of salts in the roots might be the cause when the plants had been on distilled water and addition of salts to the medium had a favourable effect.

However the fact that just the same happened with plants that could take up salts, indicates that others factors must be involved. It seemed probable that a decrease of the metabolic activity caused by depletion of substances in the roots would be concerned. It has been shown that the exudation could be promoted by the addition of sugar to the medium (fig. 11). It therefore appeared plausible to suppose, that deficiency of sugar would be the cause of this decrease. It appears from table 13 (experiment 24) that exudation and salt secretion increased when sucrose was administered to a plant that was still exuding weakly, even though the addition of sucrose caused an increase in the osmotic value of the medium. This stimulation, however, was only temporary: after about one hour the exudation decreased again, and less salt was given off. When a plant was put on a salt solution containing 0.02 M sucrose immediately after decapitation, a considerable exudation could be observed over two days, the course of which was just the same as when no sucrose was administered (experiment 24b). Periodic fluctuations in exudation, salt secretion and k also occurred when the plants were decapitated right over the two lowest leaves, so that these could be submerged in a sucrose solution (experiment 24c). Thus we did not succeed in demonstrating any influence of the sugar supply on periodicity, which corresponds with WENT's results (1944).

The experiments described up to now were carried out with plants that had been in the light by day and in darkness during the night.

In later experiments however plants were used, that had been illuminated overnight, while they were kept in the dark during the day. We see from experiment 24*d* (table 13) that these plants also show a maximal exudation, salt secretion and conductivity for water by the end of the morning. So in contrast to GROSSENBACHER (1939) we find no influence of the period of lighting, except if it might be that the rhythm is fixed at an early stage of development; for as we have said, the plants were in an ordinary green-house during the first 4-6 weeks; after that they were kept in artificial light at night for 5-6 weeks.

The diurnal fluctuations of these plants were usually much smaller. In particular the exudation showed strikingly small changes during the first day after decapitation. Only in the afternoon did the exudation begin to decrease. A distinct maximum could not be observed, although the average exudation was stronger by day than by night. Next day however, the fluctuations were more obvious. This might indicate that the periodical changes are not a normal phenomenon, but that they are somehow caused by decapitation or one of its after-effects. It was necessary now to investigate whether perhaps the moment of decapitation might determine the time at which the maxima occurred. The plants were usually decapitated the evening before the experiment was to take place. When they were cut off at 8 a.m., however, the exudation proved to increase gradually during the first day (table 13, experiment 24*d*); next day, that is a good 24 hours after decapitation, they showed a maximal exudation at precisely the same time as plants that had been decapitated 15 hours earlier. So we could not confirm the results of SPEIDEL, HEIMANN and GROSSENBACHER.

We have already pointed out that the tissue exudation at any rate shows periodic fluctuations (experiment 24*a*). It may be questioned whether the same holds true for the part of the exudation that depends on the uptake of salts. This was investigated by determining the salt effect at various times of the day. For this purpose the plant was transferred from a Hoagland solution to a solution of mannitol. The solutions were changed again after half an hour. However, it proved to be difficult to gain a proper insight into the changes of the uptake secretion. This must be determined by subtracting the amount of salt given off per unit of time on a solution lacking salts from the quantity that is given off when salts are present in the medium. These values cannot be determined at the same time. When as in this case the tissue secretion is very variable, incorrect values must be obtained for the uptake secretion. For this reason the uptake secretion was calculated in the following way: we determined the total quantity of salts given off immediately before the substitution of a mannitol solution and half an hour after the mannitol solution had been changed again for a Hoagland solution. From those data we estimated the value that the total salt secretion would have had at the moment at which the tissue secretion was determined. However this method is not very accurate either. The data obtained in this way are given

in table 14. They indicate that the uptake secretion also changes, but the changes are not very evident.

The composition of the sap proved to change little during the day (table 6). This corresponds with the fact that the osmotic value of the sap was not very variable. The amount of phosphate given off changes comparatively more than that of nitrate, but we must consider

TABLE 14
Periodic changes of the salt secretion

Experiment	time	amount of salt given off on a Hoagland solution	amount of salt given off on a mannitol solution (tissue secretion)	uptake secretion
25	9.15	0.68		
	9.50		0.55	0.15
	10.25	0.71		
	11.55	0.65		
	12.30		0.41	0.19
	13.05	0.54		
	14.25	0.52		
	15.00		0.32	0.15
	15.35	0.41		
	16.40	0.35		
	17.15		0.20	0.08
	17.50	0.21		
25a	9.20	0.49		
	9.50		0.35	0.18
	10.20	0.57		
	12.00	0.63		
	12.30		0.42	0.20
	13.00	0.61		
	14.30	0.63		
	15.00		0.37	0.25
	15.30	0.61		
	16.45	0.48		
	17.15		0.24	0.18
	17.45	0.35		

the possibility that in this case the error of determination might have some influence since the concentration of phosphate was sometimes very small.

The exudation of *Sanchezia nobilis* proved to be much more regular over a longer time. This has already been seen in table 6 (experiment 10a): both quantity of sap and of salts given off per unit of time changed very little over 24 hours. In some cases greater fluctuations were observed. In experiment 24e (table 13) the greatest rate observed was 26.5 mm/30 sec, the smallest 23 mm/30 sec. K decreased simultaneously from 778 to 622. These changes are much smaller than those observed with tomato plants (table 14). Rate of exudation, salt secretion and k were proved to change to approximately the same extent.

These plants were grown under the same conditions as the tomato

plants, that is in the lighted green-house. According to HEYL diurnal fluctuations in the exudation of *Sanchezia* can be attributed to changes in the temperature of the surroundings. This could not be the case here; even though the temperature of the room might change a little; for the temperature was not at a maximum at noon, but at the end of the afternoon, a time when the exudation was already decreasing again. Moreover changes in air temperature of only $\pm 2^{\circ}\text{C}$ were concerned. So we have not succeeded in finding a relation between some external condition and periodicity. A more extensive investigation would, however, be required to decide whether the periodicity of these plants cannot in fact be influenced by external conditions.

IV. GENERAL DISCUSSION

When planning this investigation we started from the working hypothesis that the exudation process would consist of an active transport of salts to the vessels and an osmotic transport of water caused by the resulting difference between the osmotic values of the xylem sap and the outer solution. If this is true two internal factors should control the exudation: the secretion of salts into the vessels and the water conductivity of the roots. The latter factor, naturally, influences the transport of water and in that way the concentration of the sap in the vessels.

We will first discuss these factors separately in the light of our experimental results and afterwards we will try to give a picture of the exudation process as a whole.

Salt secretion

In section 1 we have argued that the exudation might consist of two parts: that caused by salts given off from the tissue and at the same time the additional transport of water caused by salts taken up from the medium and given off to the vessels. These conclusions were drawn from the following facts: the course of the change in the rate of exudation indicated that less salt is given off to the vessels immediately the uptake of salts is inhibited, but nevertheless that exudation and salt secretion may continue at a slower rate for a long time. This part of the exudation and secretion could be influenced by changes in the osmotic value of the medium, while so far as the exudation resulting from salt uptake was concerned, only the transport of water proved to be so dependent, the transport of salts being influenced only by the salt concentration of the outer solution.

We will first be concerned with the case when no salt is taken up from the medium, so that only the tissue secretion is active. It will be remembered that the expression tissue secretion is used for the whole process concerned with the transfer of salts from the tissue into the vessels.

It is known that salts can be carried from the roots to the shoot

when plants are on distilled water (STEWART, HARRISON and PREVOT, 1942; HOAGLAND 1948). The salt content of the tissue is much decreased in such a case. The plasm is usually supposed to contain few free ions (ARISZ 1945). As it seems improbable that ions like nitrate and chloride would be present in large quantities in a bound state, we must assume that ions will be given off from the vacuole. It is possible that with phosphate the situation is different. HYLMÖ (1953) concluded that in the roots of intact pea plants calcium would leak from the vacuole and be transported to the shoot, when the medium consisted of distilled water.

Little is known of the way in which salts are given off from the vacuole. Salts that have been accumulated in the vacuole are generally supposed to be given off with some difficulty (HOAGLAND 1948). It seems probable that the rate at which these salts are given off will control the magnitude of the tissue secretion.

We found a decrease in the exflux of salts from the tissue when the osmotic pressure of the medium was enhanced. The reason for a relation between the osmotic value of the medium and the rate at which salts are given off from the cells, is not very obvious. LUNDEGÅRDH (1945) gave the following explanation: when salts are given off by a cell, its osmotic value decreases, so that water is exuded at the same time. This exudation of water from the cells is determined by their turgor pressure, which depends in its turn on the osmotic pressure of the medium. A smaller quantity of this salt solution is pressed out when the turgor pressure is less. It seems plausible to seek the answer to this problem along these lines, that is by supposing that a salt solution is given off from the tissue. It must be understood however, that there remain some differences between our view and that of LUNDEGÅRDH. It has been demonstrated in the experiments of ARISZ, HELDER and VAN NIE (1950, 1951) as well as in our own, that the water movement is osmotically regulated even when no salts are present in the medium. The amount of sap exuded decreases, while its osmotic value increases as the osmotic value of the medium is enhanced. This regulation is very rapid. This indicates that if a salt solution is given off from the tissue, more water must be sucked in from the medium as a result of the concentration difference between this salt solution and the outer solution. LUNDEGÅRDH did find a decrease in the rate of exudation but no change in the concentration of the sap. For this reason he concluded that the solution was given off in a smaller quantity, but that the relation between salt and water was unchanged.

LUNDEGÅRDH supposed that only the vascular epithelium would exude a salt solution. It seems however more plausible to suppose that salts are given off from the whole root tissue. It has been shown in the experiments with inhibitors that the exudation was quickly decreased by a considerable amount, but that it took a much longer time before the tissue secretion had been fully inhibited. This could be considered as an indication that regions towards the periphery of the roots as well as the more central parts are of importance for the salt secretion. The fact that the tissue secretion is fully inhibited later

than the uptake secretion might also be explained in this way. It seems questionable, however, if this can be considered as sufficient evidence, since it is known that various inhibitors can permeate very rapidly.

There are few data on the way in which salts are transferred from the tissue to the vessels. The theories that have been advanced on this subject are mostly concerned with the case when salt is taken up from the medium. They will be discussed below.

We supposed that salts after having been taken up from the outer solution, could be transported directly to the vessels. This supposition is not a new one. HOAGLAND (1940) has already pointed out its possibility. BROYER (1950) found in his investigation on the uptake of radioactive bromide by high salt roots, that the exudation sap contained more radioactive bromide than the sap expressed from the roots. From this he concluded that in such circumstances bromide might migrate to the vessels through the cytoplasm circumventing the vacuole. ARISZ (1948, 1952) also came to the conclusion that in the cells of leaves of *Vallisneria spiralis* chloride ions could be transported through the plasm without having been first accumulated in the vacuole. This process was not affected by KCN, nor by DNP.

We found that the exudation — and thus the salt secretion into the vessels — increased within a few minutes, if a solution lacking salts was replaced by a solution of KCl or KNO_3 . It may be questioned whether this means that within that time enough ions taken up from the medium have reached the vessels to cause an apparent increase in the exudation. It appeared from determinations of the composition of the sap, that in similar cases the increase of the exudation must be attributed to a secretion of chloride or an increased secretion of nitrate. It is not possible however, to put this fact beyond dispute. It might be that the increase in the rate of exudation is due at first to a larger secretion of ions already present in the root. It has generally been found, that the transfer of salts across the roots takes rather more time. CRAFTS and BROYER (1938) found bromide in the exudation sap of squash plants half an hour after it had been added to the medium. As the transport of salts from the roots to the cut surface would take approximately 10 minutes according to their calculation, 20 minutes would be required to bring salt from the medium into the vessels. LUNDEGÅRDH (1949) found that changes in the salt concentration of the medium became evident in the sap only after a quarter of an hour. In his experiments on uptake and transport of neutral red he stated that the dye was first accumulated in the vacuoles of the cortical cells. Afterwards it disappeared, and it could be found in the walls of the vessels within half an hour. In LUNDEGÅRDH's opinion this would indicate the way in which ions are transported through the roots. The fact that the dye was first accumulated in the vacuoles indicates that the tissue secretion was concerned here. We pointed out in section 3*b* that there are indications that not all ions are transported to the vessels in the same way. It follows from this that it is not

possible to apply uncritically data on the transport of a certain substance to the transport of all ions.

Part of the salt taken up must go to the xylem vessels. It has, however, been shown that accumulation in the tissue can take place at the same time. HOAGLAND (1948) found the same. It may therefore be asked whether in the case of salt uptake from the medium salts can be accumulated in and given off from the vacuole at the same time. It might also be possible, that in such circumstances all nitrate given off to the vessels comes from the medium. The tissue secretion would then only begin when the supply of salts from the outer solution was cut off. It is difficult to decide this question. It has been shown by determinations of the salt content of the sap that nitrate and chloride can be given off at the same time, while only chloride is taken up. This is, however, no proof that the same ion can at the same time be transported directly to the vessels and also given off from the cells. There is however a more indirect indication that this is actually true. We have demonstrated that only the tissue secretion decreases when the osmotic pressure of the medium is enhanced. The fact that less salt is given off to the vessels when the osmotic value of a salt solution is enhanced (table 2 and experiment 16) indicates that the tissue secretion must be active. The decrease of the salt secretion may be so great that a decrease in the secretion must be involved, since the other salts are quantitatively unimportant.

Both uptake and tissue secretion were shown to decrease under the influence of inhibitors. This suggests that these processes coincide at least partially. Where they do so could be in the transport of salts through the symplast of the roots to the vessels.

Administering sugar proved to promote the exudation only when salts were present in the outer solution. We mentioned the possibility that sugar promotes the uptake of salts in the symplast. It proved however, to take some time before the effect of sugar was perceptible. This might indicate that sucrose must be taken up into the tissue before it can act on the exudation. In that case a promotion of the transport should rather be expected. It therefore remains remarkable that the tissue exudation was not affected when the outer solution did not contain salts, but it might be that in this case the rate at which salts are given off by the tissue controls the salt secretion.

LUNDEGÅRDH (1950, c.f. BURSTRÖM 1951) advanced a theory on the transport of salts to the vacuole and to the vessels. According to his idea anions should be transported across the cytoplasm along a bridge of cytochromesystems. Oxidation should take place at one end of the bridge (the cell surface), reduction at the other (vacuole or vessels), so that anion respiration would be of importance. WEEKS and ROBERTSON (1950) developed a similar idea.

The inhibition of salt secretion by KCN would be consistent with the supposition that the cytochromesystem is concerned with the transport of salts. However according to the theory just mentioned transport of ions depends on the anion respiration, which operates only if salts are present in the medium. In our experiments a notable amount

of salt proved to be transported to the vessels when salts were lacking in the outer solution. This transport was also affected by KCN. We have pointed out already that it seems more probable that the inhibitors act more indirectly on the exudation.

LUNDEGÅRDH's theory cannot account for the differences in the transport of the various ions (nitrate and chloride on the one hand, phosphate on the other). HELDER (1952) thinks that ions may be bound to a certain carrier, perhaps in connection with metabolism (c.f. STEINBACH). He supposes, however, that transport might take place by the cytochromesystem, when the salts have entered the plasm. It remains questionable if this can explain the differences observed here.

Phosphate seems to be retained in the tissue to a greater extent than nitrate and chloride, as is indicated by the fact that it takes so much time before phosphate that has been taken up reaches the vessels. This is consistent with the supposition that phosphate will rapidly be bound chemically in the protoplasm. ARISZ (1952) even points to the possibility that phosphate might be transported in a bound state.

We have mentioned the view of CRAFTS and BROYER concerning the process of salt transport and exudation. WIERSUM (1947) thinks that cells within the endodermis have as the result of lack of oxygen a lower accumulation level than the cells at the surface of the roots, so that leakage of salts to the vessels should occur. ARNOLD (1952) like LUNDEGÅRDH believing that each cell takes up salt and gives it off, so that there is no transport through the symplast, thinks that salts are given off from the cells by an active process, controlled by glycolysis. This opinion is founded on a hypothesis of LUNDEGÅRDH (1949). LUNDEGÅRDH himself revoked this supposition (1950). According to his opinion salts are exuded passively from the cells. This process is not connected with metabolism, but it can occur rapidly because of the high salt permeability of the tissue. By this is meant a kind of exchange at the cellular interfaces, which might at the same time explain why only salts, and not organic substances, are given off to the vessels. HOAGLAND (1948, c.f. BIDDULPH 1951) calls the process that controls the exflux of salts into the vessels "a process akin to secretion". ALBERDA (1948) also thinks that a secretion process is concerned: the salt deficiency caused by a secretion of salts into the vessels would cause the transport of salts in that direction. ARISZ, HELDER and VAN NIE compare this process with the secretion of salt by the salt glands of *Statice*. ARISZ (1953) succeeded in demonstrating that the accumulation of chloride in the vacuole of the leaf cells of *Vallisneria* was inhibited by DNP, but the uptake from the medium by KCN, while the transport through the protoplasm was affected in neither case. This implies that different active processes are concerned with uptake into the plasm and secretion into the vacuole, but we do not know if the same holds true for the secretion into the vessels. Considering the transpiration stream as a passive mass flow from medium to shoot through cell walls and cytoplasm

HYLMÖ (1953) gives the following working hypothesis of the exudation process: he supposes that with the development of the interconnecting vessel elements in the xylem the plasm surfaces continue to function for some time when the transverse upper wall has been ruptured. This xylem cell would accumulate ions in the same way as the other cells of the root tissue. The plasm, probably the tonoplast, functions as a semipermeable membrane. Thus there is no secretion into the xylem but the xylem elements take up ions, which causes a passive flow of water.

From our own experiments we can only conclude that it seems less probable that there is a leakage of salts to the vessels along a concentration gradient, since it would then be difficult to explain the sudden large decrease or increase of the salt secretion when salts are removed from or added to the medium, because in such circumstances some after-effect would be expected. Whether there is a secretion or an accumulation of ions into the xylem vessels we cannot yet decide.

Water conductivity

It has already been stated in the Introduction that k in the formula $b = k(O_b - O_m)$ should be considered a measure of the water conductivity of the roots. The value of k depends on several circumstances. It reflects both the size of the root system and its resistance to water transport.

K has been shown to change in several cases. This might happen under constant conditions, a case which will be discussed later. Changes of k could also be observed as the result of a change in the osmotic value or the pH of the medium, and under the influence of inhibitors. The changes were usually reversible, only inhibitors could sometimes cause an irreversible decrease of k .

A decrease of k appeared to be nearly always accompanied by a diminished exudation as well as by a reduced salt secretion. The reverse also held true.

Several authors mentioned the possibility that the resistance of the roots to water might decrease as more water was sucked through them. JOST (1916) made this supposition because it appeared from his experiments that the suction tension in the vessels resulting from the transpiration of the shoot would otherwise be improbably high. KÖHNLEIN (1930) while criticizing JOST's methods, came to the same conclusion in a different way. BREWIG (1937, 1939) demonstrated that the apical zones of roots of *Vicia Faba* were more permeable to water than the basal parts, but the resistance of the basal parts appeared to decrease more if the suction tension in the vessels increased. These results were confirmed by BROUWER (1953). LUNDEGÅRDH (1945) found that the quantity of water sucked through wheat roots did not increase proportionately to the suction force but to a greater extent. At first he supposed valves to be present which would open at a certain pressure, but later (1950) he stated that the cortex would let pass much more water when the intercellular channels did not contain air any more but were filled with water. It would take

some time before all air would be gone; from that instant, however, water could pass more easily.

These decreases in the resistance to water flow which mean an increase in the water conductivity of the roots, were always accompanied by an increase of the suction tension in the vessels. (BREWIG, BROUWER). In our experiments rate of exudation and water conductivity increased but there was no rise of the suction tension. The suction tension in the vessels even decreased when the osmotic value of the medium was lowered, while k nevertheless increased. When k changed owing to periodicity or the effect of inhibitors the suction tension remained unaltered. This indicates that in spite of the apparent similarity we have been examining a different phenomenon to that discussed by the authors quoted above.

The fact that changes of salt secretion and k run parallel might indicate that these two are interdependent. This would be so, for instance, if salts and water were given off together to the vessels by one process which could be influenced by internal and external conditions. In that case it would not be possible to take k as a measure of the water conductivity. We have, however, seen that under certain conditions, viz. when there is a reduction of the salt secretion as the result of a decrease in the salt concentration of the medium k remains unaltered, or even increases (when a Hoagland solution is replaced by distilled water). Conversely it appears possible to diminish k by lowering the pH of the medium without the salt secretion changing considerably. From this we can conclude that the salt secretion and k are not necessarily interdependent. The cause of their simultaneous change is to be sought in a change of a process that influences both or that both possess in common (such as transport through the cells). In section 6 we have already concluded that inhibitors might affect both salt secretion and water conductivity by acting on metabolic processes important to the state of the protoplasm, for which there is a parallel in the phenomenon concerned with oxygen deficiency (HOAGLAND and BROYER 1942; BROYER 1951). The last case of simultaneous changes of k and salt secretion resulting from changes in the osmotic pressure of the medium is less clear. We have seen that if we follow LUNDEGÅRDH's idea the decrease of the salt secretion would be connected with a decrease of the turgor pressure of the tissue. BROUWER's experiments, however, would indicate that in such circumstances the conductivity for water should rather show an increase. It may be observed, however, that the changes of turgor pressure in BROUWER's experiments must have been much greater than in ours.

We mentioned above that the resistance to the movement of water decreases when a solution of salts is replaced by distilled water. ARISZ, HELDER and VAN NIE considered this a specific effect of the salts present in the Hoagland solution. It appears however from the experiments given in section 1, that k does not change if a Hoagland solution is replaced by another solution lacking salts but isotonic with it. The increase of k in the former case must result from the simultaneous decrease in the osmotic value of the medium. We did not

succeed in demonstrating any specific effect of ions on k . Other authors (ROSENE 1941; HAYWARD and SPURR 1943; LONG 1943) have not found any influence on the uptake of water by roots either.

As to the question of which way water moves to the vessels we may observe that the influence of inhibitors on the water conductivity points to the plasm being involved. This is confirmed by the fact that changes in the pH of the medium can also affect k . We cannot, however, decide whether only the protoplasm of the endodermis is concerned or that of the other cells of the roots as well.

We have given the following conception of the exudation process: a solution of salts flows from the root cells into the vessels. This solution attracts water from the medium osmotically. In addition salt taken up from the medium can be given off to the vessels. This also results in an osmotic water transport.

There is an objection to this idea concerned with the determinations of the osmotic values of the exudation sap. ARISZ, HELDER and VAN NIE stated that these values were usually lower than should be expected on the strength of the formula $b - k (O_v - O_m)$. We repeatedly found the same (table 2).

ARISZ, HELDER and VAN NIE mentioned various explanations for this phenomenon. In the first place experimental errors might be involved. We have discussed this possibility before. It seems rather improbable that experimental errors would be important on account of the accurate method which was used for determining the osmotic value of the exudation sap. Setting aside this possibility it might be supposed that salts are taken up from the sap in the vessels during the upward transport. In the second place it must be kept in mind that the formula just mentioned was derived starting from the supposition that the zone of the roots in which salts are given off to the vessels coincides with the water absorbing zone. If the latter is larger, the sap will be diluted as more water will be sucked in afterwards. Finally a similar deviation would be observed if water and salts were given off together.

In chapter III section 1 we have demonstrated that the osmotic value of the sap may also be higher than we should expect from the exudation observed. This might indicate that water is taken up from the sap during the upward transport. This seems rather improbable. Another explanation would be that the surrounding cells secrete salt or a concentrated salt solution into the vessels. This must happen then in the zones of the roots where osmoregulation is difficult, that is in older parts. Our difficulty is that we have to explain two conflicting facts. In both cases we pointed out that salts might be given off as a solution. It is known for instance that leaves of salt plants also secrete salt as a solution. We have already pointed to the probability of a simultaneous transport of salt and water by the process we called tissue exudation.

It is known that in excised roots most salts are accumulated in the

apical zone (PREVOT and STEWARD 1936; STEWARD, PREVOT and HARRISON 1942). STEWARD, PREVOT and HARRISON compared the accumulation of salt in excised barley roots and in the roots of intact plants. They found that in both cases most salt was accumulated in the apical zone, but much more in the first case than in the latter. This difference was greater than that in the accumulation in the basal parts in both cases. From this they concluded that the greatest quantity of salt would be given off to the vessels in the apical zone. The experiments of BREWIG and BROUWER demonstrated that the resistance to water transport is smallest in the tip zones. It is probable that the exudation is mainly localized in this part. As ARISZ, HELDER and VAN NIE already pointed out, we do not know if the zone in which most salt is given off coincides with the zone of the smallest resistance. The uptake secretion may be localized chiefly in the apical zone, but it is possible that the tissue secretion covers a larger zone, as it seems probable that the older parts of the roots give off salt accumulated before. LUNDEGÅRDH (1950) investigating the exudation of root pieces distinguished a tip zone of approximately 30 mm and a basal piece of 50 mm. The latter was divided into an upper and a lower part. The upper part of the basal piece was proved to give off more nitrate than the other parts and the exudation sap was more concentrated. This agrees with our supposition that the older parts of the roots also give off salts, while the water movement is hampered because of the higher resistance of the root. We may observe that it seems possible that in the conditions of LUNDEGÅRDH's experiments the tissue secretion predominated, as very young plants were used. This might explain some of the contrasts between LUNDEGÅRDH's experiments and our own. BROYER (1950) found a direct transport of salts to the vessels only with high salt plants. In our experiments also plants were used that had been grown under such conditions that the roots must have been in a rather high salt condition. It is not impossible that with low salt roots accumulation into the vacuole might prevail, so that little salt is available for direct transport to the vessels. The exudation of low salt plants is so weak that it is difficult to perform any experiments with them.

To gain a more thorough understanding of the exudation process it will be necessary in the first place to collect more data on the localization of the various processes concerned.

Periodicity

The periodic fluctuations of the rate of exudation proved to be accompanied by changes in the amount of salt given off and the water conductivity. Various authors had already suggested the latter possibility (SKOOG, BROYER and GROSSENBACHER 1938; GROSSENBACHER 1938). The periodic cycles in the transpiration of plants showing a maximum by day and a minimum during the night under constant conditions (MONTERMOSO and DAVIS 1942) might also be explained by assuming changes in water conductivity. The investigations of SKOOG, BROYER and GROSSENBACHER showed that more factors must

be involved. They also found fluctuations in salt secretion. The maximum occurred a little later than that of the exudation, while in our experiments both occurred at the same time.

We have ascertained that the tissue secretion at least shows a periodic fluctuation. This was, however, less obvious for the uptake exudation. If changes in the tissue secretion only are concerned, it would be attractive to connect this phenomenon with another case of a simultaneous change of tissue secretion and conductivity for water, that occurs when the osmotic value of the medium is changed. Above we discussed the possibility that the latter changes might be related to changes in the turgor pressure of the tissue: a salt solution would be expressed at a rate which varies according to the magnitude of the turgor pressure. This is interesting in connection with HAGAN's paper (1949). This author determined the negative exudation of wilted *Helianthus* plants, that is the rate at which water was sucked in through the stump. The negative exudation proved to be maximal during the night and minimal by day. In the latter case positive exudation might even occur. This could happen when the plants were in soil, and also if they were in a moist room, when no water could be taken up. The cycles disappeared if N_2 or CO_2 was present in the medium, but the negative exudation continued, although at a slower rate. HAGAN considers changes in the hydration of the protoplasm responsible. These experiments at any rate show that the root cells can express liquid. HAGAN did not investigate whether water or a salt solution was exuded.

BÜNNING (1942) demonstrated that various periodicity phenomena were accompanied by changes of turgor pressure. ENDERLE (1951) showed that the turgor pressure of carrot tissue could change periodically. According to BÜNNING this would be caused by fluctuations in the production of respirable substrate. These fluctuations would result from changes in the activity of the enzymes concerned. Changes in the intensity of the respiration would exercise influence on the enzyme activity and so on. BÜNNING (1951) found with several plants that the activity of the enzymes concerned with the synthesis of cellulose showed such fluctuations. He assumes that an endogenous rhythm is involved, which can be affected by illumination during a certain sensitive period.

SPEIDEL (1939) tried to explain the periodicity of exudation also by assuming changes in the production of respirable substrate, eventually caused by changes in the activity of enzymes. SKOOG, BROYER and GROSSENBACHER, however, found no relation between changes in exudation and respiration. SPEIDEL's hypothesis of the causes of exudation has not found much support.

If both secretion processes change periodically, the hypothesis of a fluctuating turgor pressure is insufficient. It might be that metabolic activity changes during the day, but there are few relevant data, beside those of BÜNNING and his collaborators. We may call attention to the investigation of VIRGIN (1951). VIRGIN found that the leaf cells of *Helodea densa* showed a maximal viscosity during the night, while

viscosity was minimal by day. This rhythm could be maintained over several days. Changes in the periods of illumination could influence the time at which maxima and minima occurred. VIRGIN demonstrated that the changes in viscosity ran parallel to those in permeability under certain circumstances. It seems possible to explain the changes of both water and salt transport as a result of changes in viscosity. However, there is nothing known as yet about the occurrence of such changes in the plasm of root cells.

V. SUMMARY

Starting from the supposition that the exudation is the result of a transport of salt to the vessels the salt secretion was investigated under various circumstances. For this purpose the rate of exudation was determined continuously, while the osmotic value of the sap was determined at certain instants. Also in some cases the water conductivity of the roots was determined. In most experiments tomato plants were used. Some experiments were repeated with *Sanchezia nobilis*.

The influence of the salt concentration of the medium on the salt secretion was studied first. Lowering the salt concentration of the outer solution caused a decrease of the rate of exudation, the course of the change depending on the extent to which the osmotic value of the medium had been simultaneously altered. From changes caused in this way it was concluded that the salts given off to the vessels must come partially from the root cells, while another part must have been transported directly to the vessels after having been taken up from the medium. If salts are present in the medium the latter process causes an increase in the exudation, but only if nitrates and chlorides are included. The importance of these anions for the exudation was demonstrated by determinations of the composition of the exudation sap. It appeared moreover that transport of phosphate to the vessels takes so much more time than that of nitrate and chloride that it suggests a different method of transport. The presence of nitrate or chloride in the outer solution always caused an increase in the exudation, no matter what cations were present.

The exflux of salts from the tissue — the tissue secretion — as well as the transfer to the vessels of salt taken up from the medium — the uptake secretion — was inhibited by inhibitors like potassium cyanide, dinitrophenol, sodium arsenate, sodium fluoride, iodo acetic acid and moreover phenylurethane. This indicates that both processes partially coincide. Their common part might be the transport of salts through the symplast. The water conductivity was also affected by these substances.

Sugar proved to promote the exudation only if salts were present in the medium.

The fact that the tissue secretion depended on the osmotic value of the medium led to the supposition, that salt and water are given off together from the tissue. An osmoregulation of the movement of

water must take place afterwards, the osmotic value of the sap being influenced by that of the outer solution; that is to say that water is sucked in by the solution given off from the tissue to the vessels. It was not possible to decide whether salts taken up from the medium are also transported with water. This process seemed independent of the osmotic value of the medium.

Rate of exudation, salt secretion and water conductivity of the roots were shown to suffer periodic fluctuations, a maximum occurring by day, a minimum during the night. A relation between the time at which maxima and minima occurred and certain conditions during the experiments or the period of cultivation could not be demonstrated.

The changes in the rate of exudation observed under certain conditions were in agreement with the supposition that the exudation consists of a transport of salt and a transport of water, the roots acting as an osmometer. A less satisfactory agreement between the osmotic value of the sap calculated according to this hypothesis and the value determined was sometimes found. In most cases too low a value was actually found, in some, however, the osmotic value of the exudation sap was higher than would be expected. The possible causes of this deviation have been discussed.

LITERATURE CITED

- ALBERDA, Th. 1948. The influence of some external factors on growth and phosphate uptake of maize plants of different salt conditions. *Rec. Trav. bot. néerl.* 41 : 541—601.
- ANDEL, O. M. VAN. 1952. Determinations of the osmotic value of exudation sap by means of the thermo-electric method of Baldes and Johnson. *Proc. Kon. Ned. Akad. Wet. Ser. C* 55 : 40—48.
- ANDEL, O. M. VAN, W. H. ARISZ and R. J. HELDER. 1950. Influence of light and sugar on growth and salt intake by maize. *Proc. Kon. Ned. Akad. Wet.* 53 : 159—71.
- ARISZ, W. H. 1945. Contribution to a theory on the absorption of salts by the plant and their transport in parenchymatous tissue. *Proc. Kon. Ned. Akad. Wet.* 48 : 420—46.
- ARISZ, W. H. 1947. Uptake and transport of chlorine by parenchymatic tissue of leaves of *Vallisneria spiralis*. II. Analysis of the transport of chlorine. *Proc. Kon. Ned. Akad. Wet.* 50 : 1233—45.
- ARISZ, W. H. 1948. Uptake and transport of chlorine by parenchymatic tissue of leaves of *Vallisneria spiralis*. III. Discussion of the transport and the uptake. Vacuole secretion theory. *Proc. Kon. Ned. Akad. Wet.* 51 : 25—33.
- ARISZ, W. H. 1952. Transport of organic compounds. *Ann. Rev. Plant Physiol.* 3 : 109—30.
- ARISZ, W. H. 1953. Active uptake, vacuole-secretion and plasmatic transport of chloride-ions in leaves of *Vallisneria spiralis*. *Acta Bot. Neerl.* 1 : 506—15.
- ARISZ, W. H. 1953. Absorption and transport by the tentacles of *Drosera capensis*. V. Influence on the transport of substances inhibiting enzymatic processes. *Acta Bot. Neerl.* 2 : 74—108.
- ARISZ, W. H., R. J. HELDER and R. VAN NIE. 1951. Analysis of the exudation process in tomato plants. *Jour. exp. Bot.* 3 : 257—97.
- ARNOLD, A. 1952. Ueber den Funktionsmechanismus der Endodermiszellen der Wurzeln. *Protoplasma* 41 : 189—211.
- BALDES, E. J. and A. F. JOHNSON. 1939. The thermo-electric osmometer; its construction and its use. *Biodynamica* 2 (47) : 1—11.

- BIDDULPH, O. 1951. The translocation of minerals in plants. *Mineral Nutrition of Plants*. Univ. of Wisconsin Press : 261—75.
- BLACKMAN, V. H. 1921. Osmotic pressure, root pressure and exudation. *New Phytol.* 20 : 106—15.
- BONNER, J. 1950. Arsenate as a selective inhibitor of growth substance action. *Plant Physiol.* 25 : 181—4.
- BONNER, W. D. and K. V. THIMANN. 1950. Studies on the growth and inhibition of isolated plant parts. III. The action of some inhibitors concerned with pyruvate metabolism. *Am. Jour. Bot.* 37 : 66—75.
- BRAUNER, L. und M. HASMAN. 1952. Weitere Untersuchungen über den Wirkungsmechanismus des Heteroauxins bei der Wasseraufnahme von Pflanzenparenchym. *Protoplasma* 41 : 302—26.
- BREWIG, A. 1937. Permeabilitätsänderungen der Wurzelgewebe, die vom Spross beeinflusst werden. *Zeitschr. Bot.* 31 : 481—540.
- BREWIG, A. 1939. Auflösung leichter Wasserdurchlässigkeit an Wurzeln von *Vicia Faba*. *Planta* 29 : 341—60.
- BROUWER, R. 1935. Waterabsorption by the roots of *Vicia Faba* at various transpiration strengths. I Analysis of the uptake and factors determining it. *Proc. Kon. Ned. Akad. Wet. Ser. C* 56 : 106—15.
- BROUWER, R. 1953. Waterabsorption by the roots of *Vicia Faba* at various transpiration strengths. II. Causal relation between suction tension, resistance and uptake. *Proc. Kon. Ned. Akad. Wet. Ser. C* 56 : 129—36.
- BROYER, T. C. 1950. Further observations on the absorption and translocation of inorganic solutes using radio-active isotopes with plants. *Plant Physiol.* 25 : 367—76.
- BROYER, T. C. 1951. Exudation studies on the water relations of plants. *Am. Jour. Bot.* 38 : 157—62.
- BROYER, T. C. 1951. The nature of the process of inorganic solute accumulation in roots. *Mineral Nutrition of Plants*. Univ. of Wisconsin Press : 187—249.
- BÜNNING, E. 1942. Untersuchungen über den physiologischen Mechanismus der endogenen Tagesrhythmik bei Pflanzen. *Zeitschr. Bot.* 37 : 433—86.
- BÜNNING, E. 1951. Der Tagesperiodische Verlauf der Substanzproduktion und speziell der Zellulosesynthese in Beziehung zur Photoperiodischen Reaktionsweise. *Planta* 39 : 460—70.
- BURSTRÖM, H. 1951. Mineralstoffwechsel. *Fortschr. d. Bot.* 13 : 250—68.
- BURSTRÖM, H. 1951. The mechanism of ion absorption. *Mineral Nutrition of Plants*. Univ. of Wisconsin Press : 251—60.
- CRAFTS, A. S. and T. C. BROYER. 1938. Migration of salts and water into xylem of the roots of higher plants. *Am. Jour. Bot.* 25 : 529—35.
- CRAFTS, A. S., H. B. CURRIER and C. R. STOCKING. 1949. Water in the physiology of plants. *Chron. Bot. Comp.* Waltham Mass.
- DRAWERT, H. 1952. Kritische Untersuchungen zur gravimetrischen Bestimmung der Wasserpermeabilität. Ein Beitrag zum Einfluss der pH auf die Wasseraufnahme und -abgabe. *Planta* 41 : 65—82.
- EATON, F. M. 1943. The osmotic and vitalistic interpretations of exudation. *Am. Jour. Bot.* 30 : 663—74.
- ENDERLE, W. 1951. Tagesperiodische Wachstums- und Turgorschwankungen an Gewebekulturen. *Planta* 39 : 570—88.
- ENGEL, H. und I. FRIEDRICHSEN. 1951. Das Licht als Ursache periodischer Guttationschwankungen etiolierter Haferkeimlinge. *Planta* 39 : 309—37.
- ENGEL, H. und I. FRIEDRICHSEN. 1952. Weitere Untersuchungen über periodische Guttation etiolierter Haferkeimlinge. *Planta* 40 : 529—49.
- GASZNER, G. and G. GOEZE. 1934. Assimilationsverhalten, Chlorophyllgehalt und Transpirationsgröße von Getreideblättern mit besonderer Berücksichtigung der Kalium- und Stickstoffernährung. *Zeitschr. Bot.* 27 : 257—340.
- GROSSENBACHER, K. A. 1938. Diurnal fluctuation in root pressure. *Plant Physiol.* 13 : 669—76.
- GROSSENBACHER, K. A. 1939. Autonomic cycle of rate of exudation of plants. *Am. Jour. Bot.* 26 : 107—9.
- GUTTENBERG, H. von, und A. BEYTHIEN. 1951. Ueber den Einfluss von Wirkstoffe auf die Wasserpermeabilität des Protoplasmas. *Planta* 40 : 36—69.
- GUTTENBERG, H. von und G. MEINL. 1952. Ueber den Einfluss des pH-Wertes und

- der Temperatur auf die durch Heteroauxin bedingte Veränderung der Wasserpermeabilität. *Planta* 40 : 431-42.
- HAGAN, R. M. 1949. Autonomic diurnal cycles in the water relations of nonexuding detopped root systems. *Plant Physiol.* 24 : 441-54.
- HAYWARD, H. E. and W. B. SPURR. 1944. Effects of isosmotic concentrations of inorganic and organic substrates on entry of water into corn roots. *Bot. Gaz.* 106 : 131-39.
- HEIMANN, M. 1950. Einfluss periodischer Beleuchtung auf die Guttationsrhythmik. *Planta* 38 : 157-95.
- HEIMANN, M. 1952. Abhängigkeit des Blutungsverlaufes von Beleuchtung und Blattzahl. *Planta* 40 : 377-90.
- HELDER, R. J. 1952. Analysis of the process of anion uptake of intact maize plants. *Acta Bot. Neerl.* 1 : 361-434.
- HEYL, J. G. 1933. Der Einfluss von Aussenfaktoren auf das Bluten der Pflanzen. *Planta* 20 : 294-353.
- HOAGLAND, D. R. 1940. Salt accumulation by plant cells, with special reference to metabolism and experiments on barley roots. *Cold Spring Harbour Symp. on Quant. Biol.* 8 : 181-94.
- HOAGLAND, D. R. 1948. Lectures on the inorganic nutrition of plants. *Chron. Bot. Comp.* Waltham Mass.
- HOAGLAND, D. R. and T. C. BROYER. 1936. General nature of the process of salt accumulation by roots with description of experimental methods. *Plant Physiol.* 11 : 471-507.
- HOAGLAND, D. R. and T. C. BROYER. 1942. Accumulation of salt and permeability in plant cells. *Jour. Gen. Physiol.* 25 : 865-80.
- HOFMEISTER, W. 1862. Ueber Spannung, Ausflussmenge und Ausflussgeschwindigkeit von Saften lebender Pflanzen. *Flora* 45 : 138-44.
- HUMPHRIES, E. C. 1951. The absorption of ions by excised root systems. II. Observations on roots of barley grown in solutions deficient in phosphorus, nitrogen or potassium. *Jour. exp. Bot.* 2 : 344-79.
- HYLMÖ, B. 1953. Transpiration and ion absorption. *Physiol. Plant.* 6 : 333-405.
- JOST, L. 1916. Versuche über die Wasserleitung in der Pflanze. *Zeitschr. Bot.* 8 : 1-55.
- KISSER, J. 1927. Untersuchungen über den Einfluss der Nährsalze auf die Wasserabgabe, Wasseraufnahme, relativer Spross- und Wurzelmasse und die Blattstruktur. I. *Planta* 3 : 562-77.
- KÖHNLEIN, E. 1930. Untersuchungen über die Höhe des Wurzelwiderstandes und die Bedeutung aktiver Wurzeltätigkeit für die Wasserversorgung der Pflanzen. *Planta* 10 : 381-423.
- KRAMER, P. J. 1938. Root resistance as a cause of the absorption lag. *Am. Jour. Bot.* 25 : 110-13.
- KRAMER, P. J. 1940. Sap pressure and exudation. *Am. Jour. Bot.* 27 : 929-31.
- KRAMER, P. J. 1941. Soil moisture as a limiting factor for active absorption and root pressure. *Am. Jour. Bot.* 28 : 446-51.
- KRAMER, P. J. 1945. Absorption of water by plants. *Bot. Rev.* 11 : 310-55.
- KRAMER, P. J. 1949. Plant and soil water relationships. *Mc Graw-Hill Book Co.*
- LAINE, T. 1934. On the absorption of electrolytes by the cut roots of plants and the chemistry of plant exudation sap. *Acta bot. Fenn.* 16 : 1-64.
- LONG, E. M. 1943. The effect of salt additions to the substrate on the intake of water and nutrients by roots of approach-grafted tomato plants. *Am. Jour. Bot.* 30 : 594-601.
- LUNDEGÅRDH, H. 1940. Anionenatmung und Bluten. *Planta* 31 : 184-91.
- LUNDEGÅRDH, H. 1943. Bleeding and sap movement. *Ark. f. Bot.* 31 A (2) : 1-56.
- LUNDEGÅRDH, H. 1945. Absorption, transport and exudation of inorganic ions by the roots. *Ark. f. Bot.* 32 A (12) : 1-139.
- LUNDEGÅRDH, H. 1949. Growth, bleeding and salt absorption of wheat roots as influenced by substances which interfere with glycolysis and aerobic respiration. *Ann. agric. coll. Sweden.* 16 : 339-71.
- LUNDEGÅRDH, H. 1949. The effect of indol acetic acid on the bleeding of wheat roots. *Ark. f. Bot.* 1 (4) : 295-9.

- LUNDEGÅRDH, H. 1950. The translocation of salts and water through wheat roots. *Physiol. Plant.* 3 : 103-51.
- LUNDEGÅRDH, H. 1952. Properties of the cytochrome system of living wheat roots. *Nature* 169 : 1088-91.
- MACHLIS, L. 1944. The influence of some respiratory inhibitors and intermediates on respiration and salt accumulation of excised barley roots. *Am. Jour. Bot.* 31 : 183-92.
- MONTERMOSE, J. C. and A. R. DAVIS. 1942. Preliminary investigation of the rhythmic fluctuations in transpiration under constant environmental conditions. *Plant Physiol.* 17 : 472-80.
- NIE, R. VAN, R. J. HELDER and W. H. ARISZ. 1950. Ion-secretion into the xylem and osmotic regulation of exudation. *Proc. Kon. Ned. Akad. Wet.* 53 : 567-75.
- OVERBEEK, J. VAN. 1942. Water uptake by excised root systems of the tomato due to non-osmotic forces. *Am. Jour. Bot.* 29 : 677-83.
- PREVOT, P. and F. C. STEWARD. 1936. Salient features of the root system relative to the problem of salt absorption. *Plant Physiol.* 11 : 509-34.
- PRIESTLEY, J. H. 1920. The mechanism of root pressure. *New Phytol.* 19 : 189-200.
- RALEIGH, G. J. 1946. The effect of various ions on guttation of the tomato. *Plant Physiol.* 21 : 194-200.
- RENNER, O. 1915. Die Wasserversorgung der Pflanzen. *Handwörterbuch der Naturwissenschaften* 10 : 538-77.
- RENNER, O. 1929. Versuche zur Bestimmung des Filtrationswiderstandes der Wurzeln. *Jahrb. wiss. Bot.* 70 : 805-38.
- ROBERTSON, R. N. 1951. Mechanism of absorption and transport of inorganic nutrients in plants. *Ann. Rev. Plant Physiol.* 2 : 1-24.
- ROBERTSON, R. N., M. J. WILKINS and D. C. WEEKS. 1951. Studies in the metabolism of plant cells. IX The effects of 2,4-dinitrophenol on salt accumulation and salt respiration. *Austr. Jour. Sci. Res. Ser. B* 4 : 248-64.
- ROSENE, H. F. 1941. Control of watertransport in local root regions of attached and isolated roots by means of the osmotic pressure of the external solution. *Am. Jour. Bot.* 28 : 402-10.
- ROSENE, H. F. 1944. Effect of cyanide on rate of exudation in excised onion roots. *Am. Jour. Bot.* 31 : 172-4.
- SABININ, D. A. 1925. On the root system as an osmotic apparatus. *Bull. Inst. Rech. Biol. Univ. Perm.* 5 Suppl. 2 : 1-136.
- SACHS, J. 1874. *Lehrbuch der Botanik.*
- SIMON, E. W. and H. BEEVERS. 1952. The effect of pH on the biological activities of weak acids and bases. I The most usual relationship between pH and activity. *New Phytol.* 51 : 163-90.
- SKOOG, F., T. C. BROYER, and K. A. GROSSENBACHER. 1938. Effects of auxin on rates, periodicity and osmotic relations in exudation. *Am. Jour. Bot.* 25 : 749-59.
- SPEIDEL, B. 1939. Untersuchungen zur Physiologie des Blutens bei höheren Pflanzen. *Planta* 30 : 67-112.
- SPELICH, A. und A. HAMPEL. 1936. Ueber das Bluten der Wurzel im Entwicklungsgange einer einjährigen Pflanze. *Jahrb. wiss. Bot.* 83 : 406-22.
- STEINBACH, H. B. 1951. Permeability. *Ann. Rev. Plant Physiol.* 2 : 323-42.
- STENLID, G. 1949. The effect of 2,4-dinitrophenol upon oxygen consumption and glucose uptake in young wheat roots. *Physiol. Plant.* 2 : 350-71.
- STEWART, F. C., P. PREVOT and J. A. HARRISON. 1942. Absorption and accumulation of rubidium bromide by barley plants. Localization in the root of cation accumulation and of transfer to the shoot. *Plant. Physiol.* 17 : 411-21.
- STREET, H. E. and J. S. LOWE. 1950. The carbohydrate nutrition of tomato roots. II The mechanism of sucrose absorption by excised roots. *Ann. Bot. N. S.* 14 : 307-29.
- TAGAWA, T. 1935. The relation between the absorption of water by plant root and concentration and nature of the surrounding solution. *Jap. Jour. Bot.* 7 : 33-60.
- VEEN, R. VAN DER. 1950. A small greenhouse with artificial lighting for studying plant growth under reproducible conditions. *Philips techn. Rev.* 12 (1).
- VIRGIN, H. L. 1951. The effect of light on the protoplasmic viscosity. *Physiol. Plant.* 4 : 255-357.

- WARBURG, O. und W. CHRISTIAN. 1939. Isolierung und Kristallisation des Proteins des oxydierenden Gärungsferments. *Bioch. Zeit.* 303 : 40-68.
- WEEKS, D. C. and R. N. ROBERTSON. 1950. Studies in the metabolism of plant cells. VII Dependence of salt accumulation and salt respiration upon the cytochrome system. *Austr. Jour. Sci. Res. B* 3 : 487-500.
- WENT, F. W. 1944. Plant growth under controlled conditions. III Correlations between various physiological processes and growth in the tomato plant. *Am. Jour. Bot.* 31 : 597-618.
- WHITE, Ph. R. 1938. "Root pressure" — an unappreciated force in sap movement. *Am. Jour. Bot.* 25 : 223-27.
- WIERSUM, L. K. 1947. Transfer of solutes across the young root. *Rec. Trav. bot. néerl.* 41 : 1-79.
- WIELER, A. 1893. Das Blüten der Pflanzen. *Beitr. Biol. d. Pflanzen* 6 : 1-211.

SUR QUELQUES HIERACIA SUBG. PILOSELLA DES PAYS-BAS

PAR

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I. INTRODUCTION

1. TAXONOMIE

Pilosella Tausch (1828) est un des sous-genres de *Hieracium*; les frères SCHULTZ (1862), avec bon raison, l'ont considéré comme un genre isolé; VON NÄGELI et PETER (1), puis ZAHN (2, 3) ont suivi la ligne historique et l'ont subordonné à *Hieracium*, ce que nous ferons aussi dans notre étude. NÄGELI et PETER ont divisé les *Piloselloidea* en trois groupes principaux et en plusieurs sections. Pour les Pays-Bas la division suivante suffit:

Acaulia N.P.; sect. *Pilosellina* N.P.: *H. pilosella*.

Cauligera humilia N.P.; sect. *Auriculina* N.P.: *H. auricula*.

Cauligera elata N.P.;

sect. *Pratensis* Asch.: *H. aurantiacum*, *H. caespitosum*.

sect. *Cymosina* N.P.: *H. cymosum*.

sect. *Praealtina* N.P.: *H. piloselloides*, *H. bauhini*.

Les espèces citées sont toutes des espèces "principales"; les espèces "intermédiaires", c'est-à-dire les hybrides et les espèces hybridogènes, reliant les espèces principales, seront traitées aussi dans cette étude, qui sera spécialement vouée aux *Cauligera elata*, y compris leurs formes hybrides avec les autres *Piloselloidea*. L'étude minutieuse des sous-espèces ne jouera pas un rôle important ici.

Les *Piloselloidea* néerlandaises ont été traitées en détail par ZAHN et VAN SOEST (4) dans une étude collective; la situation d'aujourd'hui, un quart de siècle plus tard, a tellement changé cependant, spécialement à ce qui concerne les *Cauligera elata*, qu'il nous semble raisonnable de donner une revision, dirigée plutôt vers les faits géobotaniques que vers les faits taxonomiques.

2. GÉNÉTIQUE

On trouve mentionné généralement que les *Hieracia* sont des espèces apomictes et, vraiment, dans le sous-genre *Hieracium* cela semble être la simple vérité exacte. Dans le sous-genre *Pilosella*, au contraire, la situation est plus compliquée.

Jusqu'à un haut degré ces espèces sont manifestement apomictes, de sorte que, dans les cultures d'une espèce isolée, on ne trouvera jamais, ou peut-être presque jamais, des variations; ce sont des cultures

de clones, d'individus identiques entre eux. Cependant, en cultivant deux espèces ensemble, souvent on trouvera bientôt au milieu des parents des hybrides, formes plus ou moins intermédiaires en général. Autant qu'on sache les formes hybrides elles-mêmes sont apomictes, probablement dans un degré plus complet que ne le sont leurs parents: une nouvelle "espèce hybridogène" s'est formée, souvent de grande vitalité. Parfois on aura la bonne chance de trouver des triple-hybrides.

Ce procès d'hybridisation, loin d'être rare chez les *Piloselloidea*, n'est pas si fréquent, ni dans la nature, ni dans les cultures, qu'on trouvera des chaînes continues de l'une espèce à l'autre. Du reste, la taxonomie des chaînes discontinues se présente comme une chose bien difficile, demandant l'étude soigneuse du spécialiste.

3. GÉOBOTANIQUE

Les aires des *Acaulia* et des *Cauligera humilia* ont leur plus grande densité dans l'Europe occidentale, quoique certaines espèces ont



1

▣ *H. echinoides*

/// espèces intermédiaires



2

H. carpsitolum



3

H. cymosum



4

H. piloselloides (q. *obscurum*)

pénétré même dans l'Asie occidentale. Les *Cauligera elata*, en général, sont beaucoup plus centrées dans l'Europe centrale et orientale jusque dans l'Asie centrale, quelques espèces même habitant l'Asie orientale!

La section *Echinina* N.P., ne jouant d'ailleurs aucun rôle dans les Pays-Bas, nous donne un bon exemple d'une telle aire. La carte 1 présente, en double hachure, la partie occidentale de l'aire de *H. echinoides* Lumn., tandis que, en hachure simple, des irradiations vers l'ouest sont figurées, dues à des espèces intermédiaires ou hybridogènes, mentionnées ci-dessus; nous pourrions les donner des formules comme *echinoides-pilosella* etc. Est-ce-que ces espèces ont avancé en comparaison de l'espèce principale? Ou, est-ce-qu'elles se sont maintenues, tandis que *H. echinoides* s'y est éteint? La réponse sûre n'est pas possible, mais nous la croyons en faveur de la deuxième supposition. *H. echinoides*, dans le passé, peut avoir eu, comme plante xérophile, une plus grande



5 *H. bauchini*



6 *H. arvicola* (*piloselloides-caespitosum*)



7 *H. tauschii* (*cymosum-bauchini*)



8 *H. aurantiacum*
(plante spontanée, d'après Zahn)

extension vers l'ouest, qu'il aura atteint ensemble avec un grand nombre d'autres espèces xérophiles, dont on trouve aujourd'hui les épreuves le long des vallées fluviales du Danube, du Main et du Rhin. L'aire d'un nombre de ces xérophytes se sera retirée vers l'est après que le climat est devenu plus froid et plus humide; les espèces intermédiaires de *H. echiioides*, moins xérophiles comme celui-ci, y seront restées jusqu'à présent.

Les *Pratensina* par contre, non-xérophiles, n'évitent pas l'Europe occidentale; la carte 2, donnant l'aire de *H. caespitosum*, sera instructive à ce point de vue.

La carte 3 présente l'aire de *H. cymosum*, l'espèce principale des *Cymosina*, espèce évitant la région atlantique.

Les *Praealtina*, préférant les stations un peu herbeuses et souvent rudérales, ne sont pas des xérophytes typiques. Il paraît qu'elles s'avancent de nos jours vers la côte de la mer du Nord avec une vitesse remarquable, s'établissant sur les bords des chemins, des chemins de fer, des canaux et sur les terrains industriels. L'aire de *H. piloselloides* (grex *obscurum*) se montre dans la carte 4 et celui de *H. bauhini* dans la carte 5. DIJKSTRA a constaté que les deux espèces sont rares dans l'extrême sud du Limbourg méridional et que — fait important — elles sont très rares dans la partie voisine de la Belgique; sans doute les deux espèces ont donc pénétré dans le Limbourg du côté de l'Allemagne.

La carte 6 présente l'aire de *H. arvicola*, espèce intermédiaire de *H. caespitosum* et *H. piloselloides*, démontrant que l'aire d'une telle espèce n'est nullement déterminée *a-priori* par l'aire commune des deux "parents".

4. FLORISTIQUE

H. caespitosum (4, 5, 6), autrefois nommé *H. pratense*, est vraiment indigène dans les Pays-Bas, spécialement dans le centre et dans le nord du pays; maintenant l'espèce est en train de s'étendre dans le sud, comme en Belgique; la carte 9 donne l'aire dans les Pays-Bas.

H. aurantiacum n'est que subspontané ici; la carte 8 donne l'aire en Europe de la plante spontanée.

H. cymosum, encore inconnu dans le pays en 1927 (4), y a été trouvé une fois comme plante adventice, ainsi que quelques *Cymosina* intermédiaires.

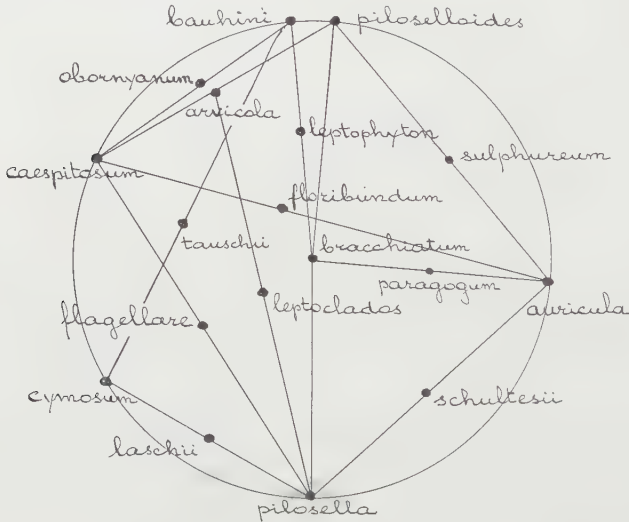
H. piloselloides (5, 6) était déjà longtemps connu de la Belgique comme plante adventice: Freyr (1867), Elsen (1874), Huy (1875), Louvain (1886). En 1930, pour la première fois dans les Pays-Bas, A. DE WEVER l'a trouvé dans le Limbourg méridional (7); depuis ce moment il s'y est étendu (9); la carte 10 donne l'aire actuelle dans le pays.

H. bauhini semble être assez rare en Belgique; ZAHN (3) ne mentionne qu'un seul endroit: Angleur; puis l'espèce a été trouvée à Chainieux, 1909 (6); peut-être elle s'est étendue dans ces derniers temps? Dans les Pays-Bas elle a été trouvée pour la première fois en 1908 dans le Limbourg méridional par A. DE WEVER, qui, en 1943 (7), publiait quelques stations nouvelles dans cette contrée. L'aire actuelle

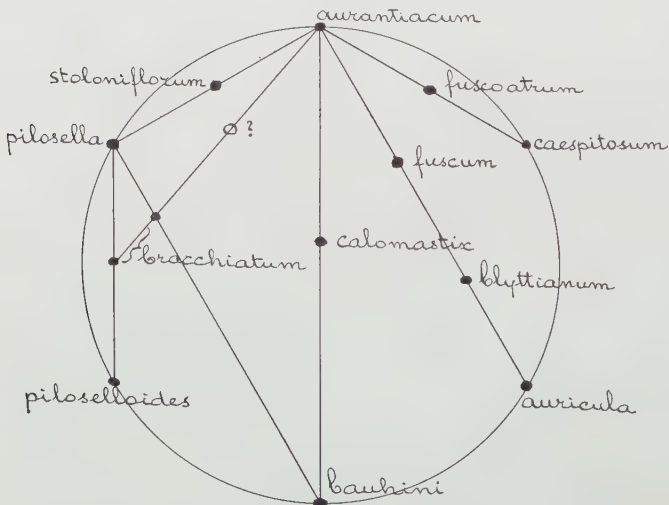
(carte 11) démontre l'expansion de *H. bauhini* dans les Pays-Bas.

Plusieurs espèces intermédiaires ont paru en même temps. Il n'y a qu'un quart de siècle qu'on ne connaît que *H. schultesii*, *H. flagellare* et *H. floribundum* (4), dont la dernière espèce est d'indigénité douteuse. Maintenant on en connaît quinze! Les relations entre ces espèces sont éclaircies dans les tableaux a et b.

Dans les paragraphes II-IV des détails plus précis sont traités.



a. Espèces intermédiaires (*H. aurantiacum* exclu)



b. *H. aurantiacum* et espèces intermédiaires

Liste des abréviations

h. = herbier

K.N.B.V. = Koninklijke Nederlandsche Botanische Vereeniging (Soc. royale botanique néerlandaise).

D. = S. J. Dijkstra — J. = Johan Jansen, † — K. = J. H. Kern —

B.R. = B. Reichgelt — Th. R. = Th. Reichgelt — v.S. = J. L. van Soest — d.W. = A. de Wever, †.

II. PRATENSINA Asch.

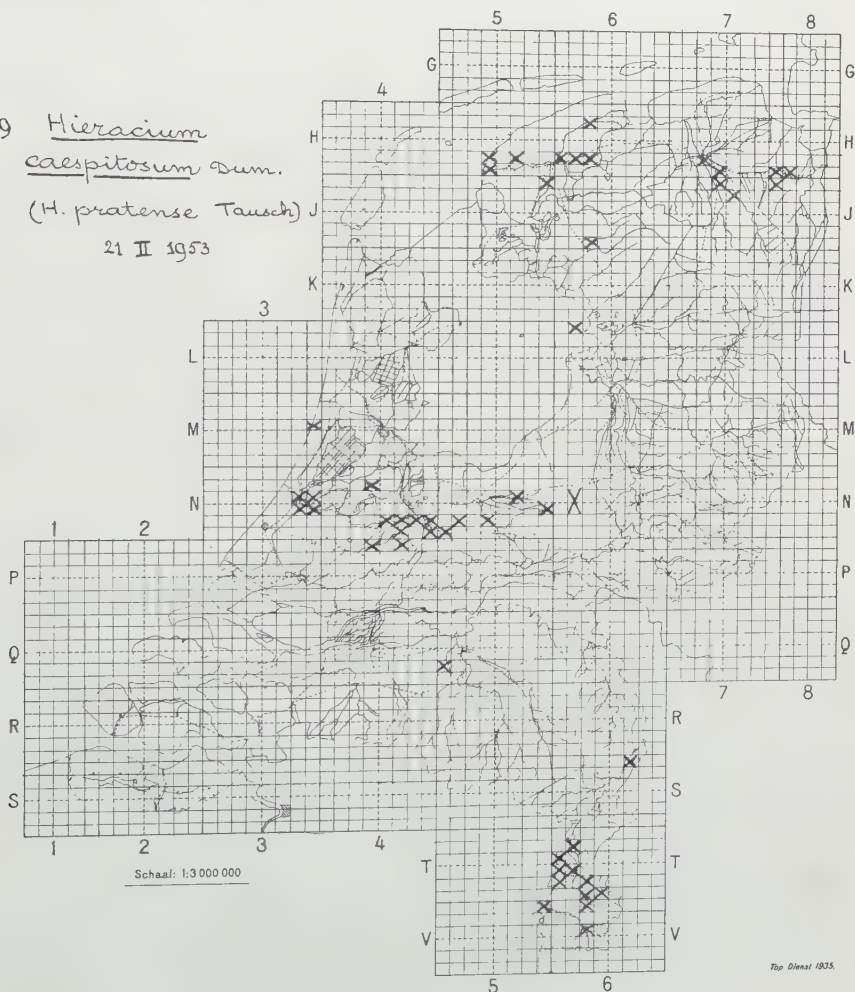
1. **Hieracium caespitosum** Dumortier (1827) = *H. pratense* Tausch (1828), carte 9.

L'aire dans le nord a peu changé; l'espèce y est assez commune. Dans le centre du pays trois extensions sont d'intérêt: Mijdrecht-Noorden, 1936 Mlle C. H. Klinkenberg, Th. Stomps (h.v.S.); Hoen-

9 Hieracium
caespitosum Dum.

(*H. pratense* Tausch)

21 II 1953



derloo-Otterloo, 1946 Balke (h.); Hoenderloo-Ugchelen, 1951 F. Hekker (h.).

Comme des plantes adventices il faut considérer: Noordoostpolder près du Voorst, 1947-'48 D. Bakker (h., h.K.N.B.V.); dunes maritimes près de Heemstede, 1949 S. E. de Jongh (h.). En outre Van Giersbergen a trouvé l'espèce près de 's-Hertogenbosch (Bois-le-Duc), 1930 (h.v.S.).

Dans le Limbourg *H. caespitosum* était seulement connu de Schinnen (1895), le long du chemin de fer, ou il croît encore. Depuis 1940 l'espèce s'est répandue dans une grande partie du Limbourg méridional: Oud-Roosteren, Buchten, Urmond, Lutterade, Limbricht, Stein, Watersleije, Brunssum, Hommert, Bunde, Wahlwijlre-Mechelen, tous observées par D.(h.) dans les années 1944-46; en 1948 Ter Linden y a été ajouté par D.(h.). Dans le Limbourg septentrional *H. caespitosum* a été trouvé depuis 1949 près de Venlo par D., K., Th.R. et v.S. (h.divers).

Dans la plupart des cas on a trouvé la ssp. *caespitosum*; la ssp. *colliniforme* N.P. est certainement beaucoup plus rare.

2. **Hieracium flagellare** Willd. (*H. caespitosum* - *pilosella*).

Cette espèce hybride s'est formée plusieurs fois comme hybride dans les Pays-Bas. Au nord du pays van Hall l'avait déjà récoltée en 1827 (4); après cela elle n'y a été jamais retrouvée. Dans le centre du pays Mlle C. H. Klinkenberg et Th. Stomps l'ont récoltée en 1936 entre Mijdrecht et Noorden, *inter parentes* (h.v.S.); puis F. Hekker (h.) l'a trouvée en 1951 avec les parents entre Hoenderloo et Ugchelen. Dans le jardin de A. W. Kloos l'hybride s'est produit spontanément.

Dans le Limbourg méridional il a été trouvé, au milieu des parents, à Brunssum, 1945 D. (h.) et près de Ter Linden, 1948 D. et v.S. (h.).

Tout le matériel séché néerlandais appartient probablement à la sous-espèce typique.

3. **Hieracium floribundum** W. et Gr. (*H. caespitosum*-*auricula*).

En compagnie d'une grande série de *Hieracia*, cette espèce intermédiaire s'est probablement produite comme hybride à Ter Linden, 1946 D. (h.). On trouve à cet endroit: *H. pilosella*, *H. auricula*, *H. schultesii* (*pilosella*-*auricula*), *H. caespitosum*, *H. flagellare* (*caespitosum*-*pilosella*), *H. piloselloides* (ssp. *obscurum*), *H. bauhini* (ssp. *weissianum*), *H. brachiatum* (*piloselloides* ou *bauhini*-*pilosella*), *H. sulphureum* (*piloselloides*-*auricula*), *H. paragogum* (*brachiatum*-*auricula* ou *piloselloides*-*schultesii*).

L'exemplaire de *H. floribundum*, récolté avec *H. paragogum*, ne permet pas une détermination tout-à-fait sûre; les poils floconneux manquent presque entièrement sur la face inférieure des feuilles, ce qui démontre que *H. pilosella* n'a pas entré dans cette hybridisation.

4. **Hieracium aurantiacum** L. et ses hybrides.

Cette espèce, souvent cultivée et subspontanée dans tout le pays (4) ne produit que rarement des hybrides ici, quoiqu'on trouve *H.*

aurantiacum assez souvent en compagnie de *H. pilosella* etc. Il faut mentionner néanmoins quelques exceptions (planche b).

La plupart des hybrides trouvés est concentrée à un seul endroit, très riche en *Hieracia*: le terrain de l'ancienne tuilerie à Brunssum. On y connaît: *H. pilosella*, *H. auricula*, *H. caespitosum*, *H. flagellare* (*caespitosum-pilosella*), *H. bauhini* (ssp. *viscidulum*), *H. brachiatum* (*bauhini-pilosella*), *H. obornyanum* (*bauhini-caespitosum*).

En 1950 D. (h.) a trouvé des plantes à cet endroit, tenant le milieu entre *H. caespitosum* et *H. aurantiacum*: c'est le *H. fusco-atrum* N.P.; *H. aurantiacum* a été découvert non loin de cet endroit et en 1951 *H. aurantiacum* croît au même endroit.

En 1951 D. (h.) y a trouvé: *H. stoloniflorum* W. et K. (*aurantiacum-pilosella*) et *H. calomastix* N.P. (*aurantiacum-bauhini*).

En 1952 D. (h.) y a récolté de nouveaux hybrides: *H. fuscum* Vill. ssp. *fuscum* (*aurantiacum* > *auricula*), *H. blyttianum* Fr. ssp. *chrysochroum* N.P. (*aurantiacum-auricula*) et deux autres formes hybrides et critiques, l'une des deux répondant à la formule *stoloniflorum-pilosella* (cfr. *H. stoloniflorum* ssp. *hausmanni* (Rchb.) N.P.) et l'autre probablement à la formule: *pilosella-aurantiacum-piloselloides*, peut-être formée comme hybride de *H. stoloniflorum* et de *H. piloselloides*; ce triple-hybride n'a pas encore été décrit dans la littérature; il faut noter toutefois que *H. piloselloides* n'a pas encore été constaté à cet endroit, mais bien dans les environs.

C'est remarquable qu'une telle explosion d'hybrides dans un seul endroit s'est produite en si peu d'années.

Du reste il n'y a qu'une seule chose à noter: *H. calomastix* N.P. s'est formé dans le jardin de v.S. (h.) en 1941 *inter parentes*: *H. aurantiacum* ssp. *aurantiacum* et *H. bauhini* ssp. *weissianum*. Après s'être multiplié quelques fois en formes nettement identiques, cette espèce hybridogène a disparu quelques années plus tard, quand, pendant la guerre, le jardin ne pouvait plus être entretenu.

III. *CYMOSSINA* N.P.

1. *Hieracium cymosum* L. et *H. laschii* (Schultz fr.) Z.

Dans son jardin à Nuth A. de Wever avait une culture de *H. cymosum*; après la mort de d.W., D. (h.) a trouvé l'espèce en 1952 à Schurenberg, non loin de Nuth. Il est bien possible que ces plantes se sont échappées de ce jardin. Il s'agit probablement de la ssp. *cymigerum* (Rchb.) N.P., qui a atteint l'ouest de l'Europe dans la vallée du Rhin (2, 8).

C'est remarquable que D. (h.) en 1952 a trouvé au même endroit l'hybride avec *H. pilosella*, nommé *H. laschii* (Schultz fr.) Z.

2. *Hieracium tauschii* Z. (*H. bauhini-cymosum*), carte 7.

En 1942 Johan Jansen (h.) a trouvé *H. tauschii* en 4 exemplaires aux bords du canal "Maas-Waal", près de Malden sur un terrain sablonneux et rudéral. Parce que ni *H. bauhini*, ni *H. cymosum* s'y trouvent, il faut conclure que l'espèce hybridogène y était introduite.

IV. PRAEALTINA N.P.

1. **Hieracium piloselloides** Vill. (3) = *H. florentinum* All. (2), carte 10.

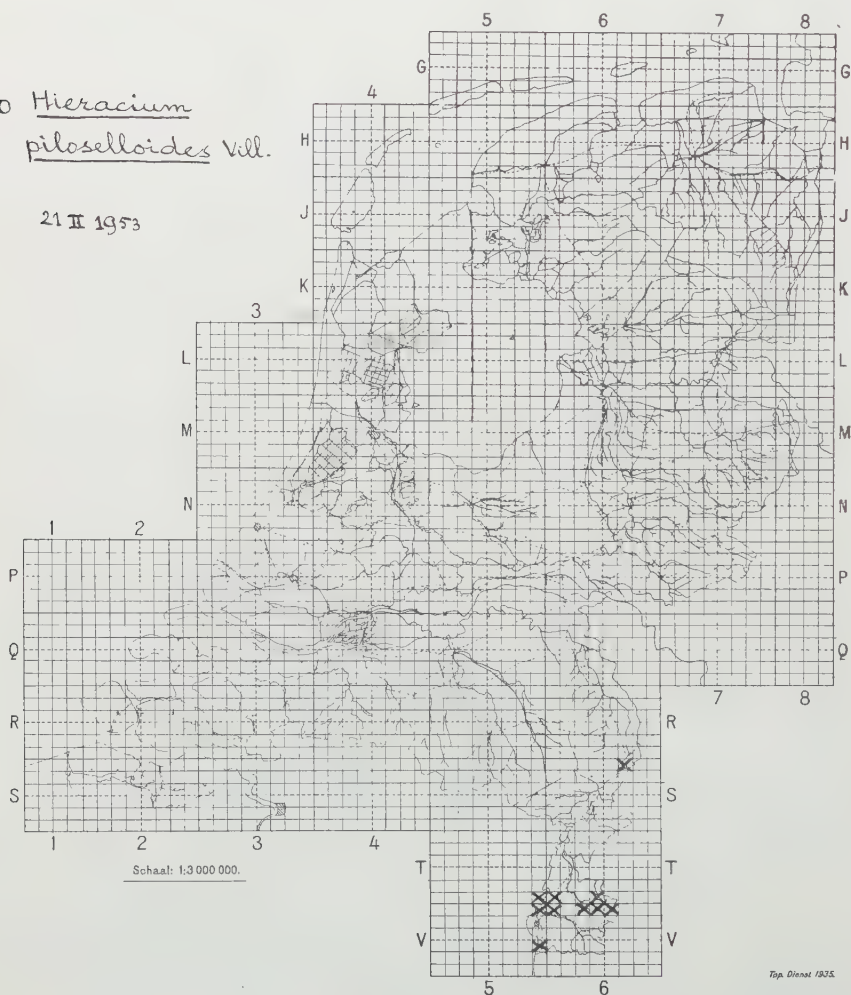
En Belgique on a trouvé la ssp. *praealtum* (Vill.) N.P., jusqu'au St. Pietersberg, Emael près de la frontière néerlandaise, 1949 D. (h.). Probablement toutes les plantes de cette espèce, trouvées dans les Pays-Bas, appartiennent à la ssp. *obscurum* (Rchb.) N.P.

En 1930 A. de Wever (h., h.v.S.) l'a trouvée, pour la première fois, à Brunssum, adventice dans la gare de triage de la mine "Hendrik".

En 1941 J. (h.), K., B.R. et Th.R. (h.) l'ont récoltée dans le Limbourg septentrional près de Venlo: Jammerdaalse heide, où elle est toujours très fréquente (h.D., h.v.S.).

10 Hieracium
piloselloides Vill.

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En 1942-'46 D. (h.) l'a constatée à plusieurs endroits du Limbourg méridional le long du canal "Juliana", p.e. à Geulle, Bunde, Elsloo; puis à Beersdal, Hoensbroek-Ter Linden, Schaesberg-Eijgelshoven, Meerssen et à la mine "Oranje-Nassau I".

Les akènes des plantes néerlandaises sont d'un brun clair, tandis que chez les formes de *H. bauhini* ils sont d'un brun-pourpre obscur. Les différences entre *H. piloselloides* et *H. bauhini* ne sont pas très grandes; l'un manque des stolons épigés, l'autre les produit d'ordinaire en abondance, sauf dans les circonstances défavorables; dans la culture *H. bauhini* immédiatement nous montre les stolons. Le caractère des stolons est probablement le seul, qui sépare nettement les deux espèces complexes. Cela nous donne dans certains cas des difficultés quand ces espèces produisent des hybrides avec *H. pilosella*, qui, lui-même, a des stolons bien développés; on ne sait plus si l'hybride répond à la formule de *H. piloselloides-pilosella* ou à celle de *H. bauhini-pilosella*; c'est bien la raison pour laquelle ZAHN n'ose pas les séparer et les réunit sous un seul nom: *H. brachiatum* Bert., qui sera traité sous no. 6. A côté de *H. brachiatum* on a distingué des formes hybrides: *piloselloides* > *pilosella* et *bauhini* > *pilosella*, qui sont nettement différentes.

2. **Hieracium sulphureum** Doell. (*H. piloselloides* × *auricula*).

L'hybride a été trouvé, *inter parentes*, à Ter Linden, 1946 D.(h.); il ne faut pas le confondre avec des formes maigres de *H. piloselloides*.

3. **Hieracium arvicola** N.P. (*H. piloselloides* × *caespitosum*).

En 1944 l'hybride a paru spontanément, entre les parents, dans le jardin de Th.R.(h.).

En 1941 K., B.R. et Th.R. (h.) ont récolté, avec *H. piloselloides* ssp. *obscurum* et *H. caespitosum* ssp. *caespitosum* à Venlo, Jammerdaalse heide, des plantes critiques de la formule *piloselloides* > *caespitosum*; en 1948 D. (h.), K., Th.R. et v.S. (h.) ont récolté au même endroit beaucoup plus de ce matériel, qui diffère de *H. piloselloides* ssp. *obscurum* dans les caractères suivants: muni parfois de stolons bien courts, la face inférieure des feuilles plus floconneuse; les folioles de l'involucre un peu poilues et plus larges. Sans doute ces plantes appartiennent à *H. arvicola*.

4. **Hieracium leptoclados** N.P. (*H. arvicola* × *pilosella*).

L'hybride de *H. arvicola* et de *H. pilosella* s'est formé à Venlo, 1947 J., K., B.R., Th.R. (h.); 1948 D. (h.), K., Th.R., v.S. (h.). Les plantes diffèrent à peine de *H. brachiatum*, montrant néanmoins quelques caractères, qui indiquent l'influence de *H. caespitosum* dans ce triple-hybride.

5. **Hieracium bauhini** Bess., carte 11.

Hors du Limbourg méridional, *H. bauhini* a été trouvé à trois stations dans les Pays-Bas: environs de Winterswijk et Kotten, 1934 Gorter (h. d'Amsterdam), 1948 C. A. Backer (h. de Leyde); Millse Peel, en masse sur un ancien champ de football dans la bruyère, 1943

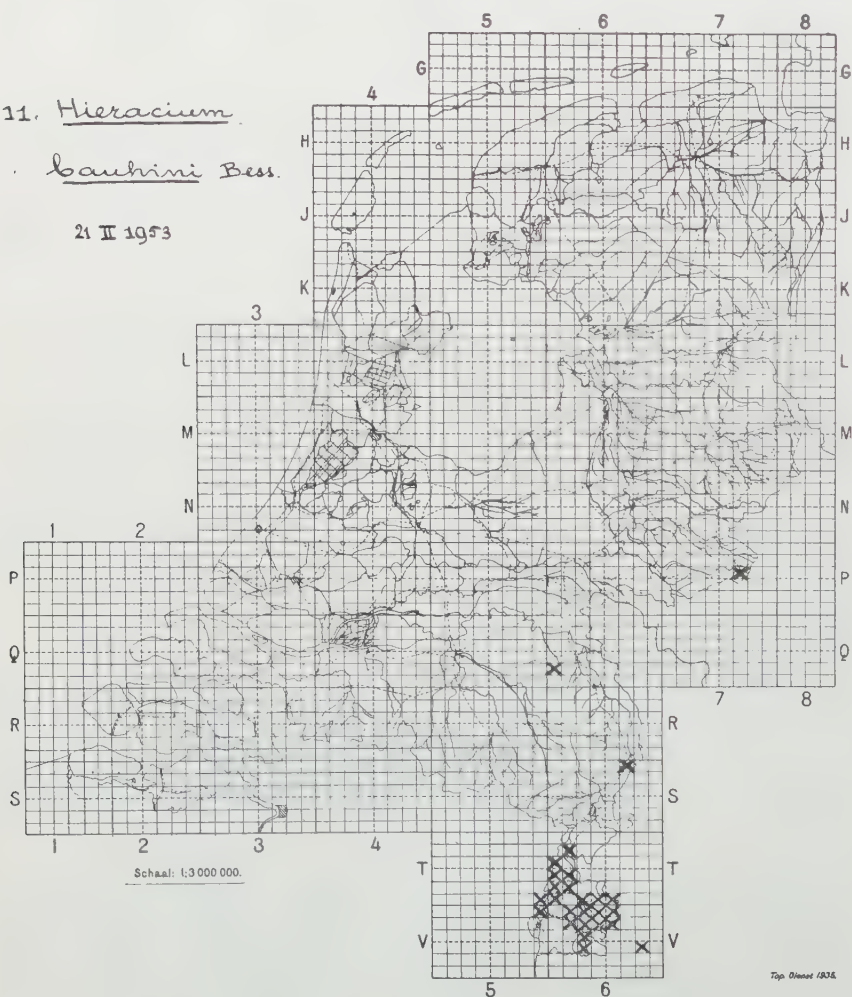
J. van den Heuvel et J. (h., h. K. & R.); Tegelen, dans une argilière, 1943 K., B.R. et Th.R. (h.).

Déjà en 1908 d.W. a trouvé l'espèce à Ter Linden le long du chemin de fer; en 1930-'32 il a trouvé *H. bauhini* dans le Limbourg méridional à plusieurs autres stations: Welsenheuvel-Sittard, Schrieversheide, Schaesberg, Geleen, Wylre, Heerlen, Meerssenhoven et Schin op Geul (h. d.W., h. v.S.). Après lui, D. (9) a constaté depuis 1943 un très grand nombre de nouvelles localités, toutes représentées dans son h.: Illenhoven, Born, Buchten, Berg, Stein, Elsloo, Geulle, Bunde, Borgharen, Nuth-Nagelbeek, Brunssum; les mines "Hendrik", "Willem Sophia", "Wilhelmina", "Oranje-Nassau I, II, III et IV"; Rimborg, Watersleijck, Puth, Geverik, Lichtenberg, Klimmen, Eijgels-hoven, Schandelen, Voerendaal; aux environs de Heerlen; Kunrade,

11. Hieracium

bauhini Bess.

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Ransdaal, ter Winselen, Kerkrade, Rolduc, Eijserbos, Beersdal, de Kakert, St. Pierrade, Boeren-Anstel, Laura, Molsberg, Valkenburg, Simpelveld, Ravensbos, Epen. En même temps K. et Th.R. (h.) ont ajouté: Eijserberg, 1948.

D. a constaté que *H. bauhini* croît aussi à l'autre coté de la frontière en Allemagne: Stolberg, Wormdal, etc.

H. bauhini est sans doute représenté dans les Pays-Bas par plusieurs sous-espèces: ssp. *weissianum* Z. en est la plus caractéristique; certainement aussi ssp. *arvorum* N.P. n'y est pas rare. La sous-espèce la plus abondante dans le Limbourg méridional est une race à pédoncules non- ou seulement très peu floconneuses. C'est un caractère typique pour le groupe *H. magyaticum* (N.P.) Z. de *H. bauhini*, dont la ssp. *besserianum* (Spreng.) N.P. a été constatée en Belgique (5) et peut-être çà et là dans les Pays-Bas. Mais le groupe *H. magyaticum*, possédant en général des stolons très longs et très fins, a une aire orientale; la race mentionnée n'y appartient pas. Elle montre souvent une autre propriété remarquable: les stolons souvent jaillissent des aisselles des tiges, caractère typique du groupe *H. aerostolonum* Z. Comme *H. magyaticum*, *H. aerostolonum* a une aire orientale et les caractères de plante xérophyle de ce groupe ne sont non plus d'accord avec ceux de la race mentionnée. Cela nous a donné des difficultés taxonomiques, jusqu'au moment où nous avons trouvé que ZAHN (2, 3) a inclus dans le groupe typique de *H. bauhini* une ssp. *viscidulum* Tausch, qui y fait une double exception, ayant souvent les pédoncules non-floconneux et les stolons jaillissants des tiges! Nous connaissons du matériel typique de cette sous-espèce dans l'herbier de Leyde.

Nous avons vu les formes "aerostolones" de la ssp. *viscidulum* de: Schin op Geul, Nuth-Nagelbeek, Oranje-Nassau II, Ransdaal, St. Pierrade, Boeren-Anstel et Heerlen-gare; la ssp. *viscidulum* est d'ailleurs beaucoup plus fréquente dans le Limbourg méridional.

6. **Hieracium bracchiatum** Bert. (*H. piloselloides* × *pilosella*, *H. bauhini* × *pilosella*).

Cette espèce intermédiaire, qui a été trouvée dans les Pays-Bas toujours en présence des parents supposés, a été trouvée hors du Limbourg-méridional: Mill, 1943 J. (h.v.S.) avec *H. bauhini*; Venlo J., K., B.R., Th.R. (h.) en 1947 et D., K., Th.R. et v.S. (h.) en 1948, en compagnie de *H. piloselloides*. D'ailleurs l'hybride s'est produit dans le jardin de Th.R. (h.) en 1943 comme *H. piloselloides* × *pilosella*.

Dans le Limbourg méridional une quantité de stations a été découverte par D. (h.): Geulle (avec *H. bauhini*), Brunssum (une fois avec *H. bauhini*, l'autre fois avec *H. bauhini* et *piloselloides*); Eijgels-hoven, Heerlen-Schaesberg et Bunde (tous avec *H. bauhini*); Ter Linden et Beersdal (tous les deux avec *H. piloselloides* et *H. bauhini*). Ensuite K. et Th.R. (h.) l'ont trouvé à Eijserberg, 1948 (avec *H. bauhini*).

Plusieurs formes ont été trouvées, dont deux sont semblables à *H. leptophyton* N.P., qui est considéré comme *H. bauhini* > *pilosella*: Eijserberg et Ter Linden (*pro parte*).

7. **Hieracium paragogum** N.P. (*H. brachiatum* × *auricula* ou *H. piloselloides* × *schultesii*).

Ce triple-hybride a été récolté à Ter Linden, 1946 D. (h.), en compagnie de *H. pilosella*, *H. auricula*, *H. schultesii*, *H. piloselloides*, *H. brachiatum* et d'autres *Hieracia*.

8. **Hieracium obornyanum** N.P. (*H. bauhini* × *caespitosum*).

Cette espèce hybridogène et intermédiaire a été trouvée en 1948 à Brunssum sur le terrain de l'ancienne tuilerie, D. (h.) et v.S. (h.); on y a trouvé deux formes, l'une d'elles bien proche de *H. caespitosum*. Tous les *Hieracia*, croissant à cet endroit, ont été cités en II. 4.

LITTERATURE CITEE

1. NAEGELI, C. VON et A. PETER, Die Hieracien Mittel-Europas, Piloselloiden. (1885).
2. ZAHN, K. H., Hieracium; Das Pflanzenreich (A. ENGLER) IV 280, (1923).
3. ZAHN, K. H., Hieracium; Synopsis der Mittel-Eur. Flora (ASCHERSON & GRAEBNER) XII. (1930).
4. SOEST, J. L. VAN, Het geslacht Hieracium in Nederland III; Nederl. Kruidk. Arch. 37: 171. (1927.)
5. SOEST, J. L. VAN, Aanteekeningen over Hieracium: Nederl. Kruidk. Arch. 44: 296. (1934).
6. SOEST, J. L. VAN, Hieraciologische Aanteekeningen in België: Biol. Jaarb. Dodonaea 4: 178. (1937).
7. WEVER, A. DE, Naar de Maastreek; Natuurhistorisch Maandbl. Limburg 32: 88. (1943).
8. TOUTON, K., Die rheinischen Hieracien I: Jahrb. Nassau Naturk. 74: 1. (1922).
9. DIJKSTRA, S. J., Floristische notities over het jaar 1944; Natuurh. Maandbl. Limburg 34: 14, 34. (1945).

ERRATA

Paper by H. J. KETELLAPPER:

p. 421, line 16: *read* or *i.s.o.* of

p. 433, table 20: *read* Pretreatment hours *i.s.o.* Petreatment hours

p. 440, line 2: *read* (page 418) *i.s.o.* (page 000)

Paper by O. M. VAN ANDEL:

p. 445, title: *read* (Received June 30th 1953) *i.s.o.*
(Received June 30th 1952)

p. 452, line 31/32: *read* $k = (b_1 - b_2) / (0_{m2} - 0_{m1})$ *i.s.o.*
 $k = (0_{m2} - 0_{m1}) / (b_1 - b_2)$

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J A A R B O E K
1953

VERSLAGEN EN MEDEDELINGEN

DER

KONINKLIJKE NEDERLANDSE BOTANISCHE
VERENIGING

OVER HET JAAR 1952

UITGEGEVEN 1 OCTOBER 1953

KONINKLIJKE NEDERLANDSE BOTANISCHE VERENIGING
AMSTERDAM

KONINKLIJKE
NEDERLANDSE BOTANISCHE VERENIGING
(Opgericht 15 Augustus 1845)

BESTUUR in 1952

Prof. Dr E. VAN SLOGTEREN, *Voorzitter.*
Dr S.J. VAN OOSTSTROOM, *Ondervoorzitter.*
Prof. Dr A.W.H. VAN HERK, *1e Secretaris.*
Mej. Dr A. JAARVELD, *2e Secretaresse.*
Dr Ir A.W. KLOOS JR, *Penningmeester,*
overleden 3 Juni 1952.
Prof. Ir J.L. VAN SOEST, *Penningmeester.*

VERSLAGEN OVER HET JAAR 1952

JAARVERSLAG van den eersten secretaris over 1952

In het afgelopen jaar heeft de Koninklijke Nederlandse Botanische Vereniging vele van haar oudere leden door den dood verloren. Het overlijden van Dr F.J.M. OFFERIJNS op 8 Januari 1952 en het overlijden van ons erelid Dr TH. WEEVERS op de 15de van dezelfde maand werden reeds in het vorige jaarverslag genoemd; uitvoerig werd geschetst, welke betekenis beide leden voor onze Vereniging gehad hebben. Ik mag daarom thans volstaan met te verwijzen naar hetgeen verleden jaar op deze plaats gezegd werd.

Op 23 Maart overleed Dr P.J.S. CRAMER, oud-hoogleraar in de Economie van de tropische landbouw voor het Indologisch Fonds te Utrecht. Hij studeerde biologie aan de Universiteit van Amsterdam; vertrok in 1905 naar Indië, waar hij bij het Departement van Landbouw werd aangesteld. In 1909 volgde zijn benoeming tot Hoofd van de Afdeling Botanische Laboratoria van dit Departement, in welke functie hij zich vooral met de koffiecultuur bezig hield. Na enige studiereizen naar verschillende gebieden van Z.O.-Azïe werd hij in 1910 benoemd tot Directeur van de Landbouw in Suriname; in 1913 keerde Dr CRAMER naar Java terug als Hoofd van het Selectiestation van het Departement van Landbouw, Nijverheid en Handel. In 1921 volgde zijn benoeming tot Directeur van het Algemeen Proefstation voor de Landbouw; in 1926 tevens tot Directeur van het Instituut voor Plantenziekten. Na zijn repatriëring in 1927 trad hij vooral op als adviseur van particuliere maatschappijen. Zeer veel heeft hij betekend voor de uitbouw van de botanische wetenschap in de tropen, het meest wel in toegepast-wetenschappelijke richting.

31 Maart verloren wij Dr J. BRAND, in leven arts te Doesburg; ruim 36 jaar was hij lid van onze Vereniging. Groot was zijn belangstelling voor de flora van ons land; vele van de jaarlijkse floristische excursies werden door hem

meegemaakt. Door zijn prettig karakter was hij alom zeer gewaardeerd. Zijn grote belangstelling voor onze Vereniging blijkt ook daaruit, dat hij zijn bibliotheek en herbarium aan onze Vereniging heeft nagelaten.

Dr H.L. VAN DE SANDE BAKHUYZEN, wetenschappelijk onderzoeker bij het Centraal Instituut voor Landbouwkundig Onderzoek, overleed 17 April te Wageningen. Hij studeerde biologie aan de Universiteit te Utrecht; daarna was hij korte tijd werkzaam bij het Rijkslandbouwproefstation te Groningen. In 1924 vertrok hij naar de Verenigde Staten van Amerika, waar hij aan het Food Research Institute der Stanford University verbonden werd. Spoedig werd hem opgedragen de ontwikkeling van tarwe onder constante omstandigheden te bestuderen; het resultaat van dit onderzoek is later in een uitvoerig boek neergelegd. In 1927 werd hij tot hoogleraar in de Botanie aan de Stanford University benoemd; in 1928 ging hij naar de State University of Arizona over. Na zijn terugkeer in ons land heeft hij zich vooral bezig gehouden met de groei en ontwikkelingsfysiologie van tarwe.

De 3e Juni verloor onze Vereniging haar oudste lid, Dr F.W.T. HUNGER, oud-directeur van het Instituut voor de Geschiedenis der Geneeskunde, Wetkunde en Natuurwetenschappen. Sedert 1895 was hij lid van onze Vereniging. Na aan de Universiteit van Leiden biologie te hebben gestudeerd, werd hij in 1899 als botanicus verbonden aan 's Lands Plantentuin te Buitenzorg. Na een korte verlof-periode, — in welke tijd Dr HUNGER privaats-docent was in de Tropische Botanie aan de Universiteit te Utrecht —, werd hij in 1906 benoemd tot Directeur van het Algemeen Proefstation op Java. Na zijn repatriëring werd hij van 1917 — 1924 toegelaten als privaats-docent in de Geschiedenis der Biologie aan de Leidse Universiteit. Nog op hoge leeftijd woonde hij de vergaderingen van onze Vereniging bij. Kort voor zijn overlijden schreef hij mij nog een voordracht over het leven van Clusius te willen houden; deze voordracht zou dan de afsluiting zijn van zijn wetenschappelijke activiteit. De dood heeft dit echter verhinderd.

Dezelfde dag leed onze Vereniging wel een zeer zwaar verlies door het overlijden van haar penningmeester en erelid Dr Ir A.W. KLOOS JR. Na zijn studie voor civiel-ingenieur te Delft was hij leraar aan de Zeevaartschool te Schiermonnikoog geworden; daarna aan de R.H.B.S. te Goes en van 1911 tot 1945 aan de Middelbaar Technische School in Dordrecht. Grote belangstelling had hij voor de levende natuur; in 1912 toetreden als lid van onze Vereniging behoorde hij al spoedig tot een van onze meest gezaghebbende en bekwaamste floristen. Grote vreugde en waardering leefden dan ook algemeen in onze Vereniging, toen de Leidse Universiteit op 10 Januari 1946 aan Ir KLOOS een eredoctoraat verleende.

Groot is het aandeel geweest, dat Dr KLOOS in ons verenigingsleven gehad heeft. Driemaal was hij penningmeester: van 1928 — 1932, vervolgens van 1938 — 1942 en in 1949 werd hij voor de derde maal met de zorg van de financiën belast. Zij die van meer nabij weten hoe zorgelijk de financiële positie van de Vereniging geweest is, maar ook weten hoeveel activiteiten zij gedurende de laatste decennia heeft kunnen ontplooien, kunnen niet anders dan bewondering hebben voor de grote bekwaamheid en zorgvuldigheid, waarmee Dr KLOOS, — en ook zijn grote vriend Dr JANSSEN —, het beheer van onze financiën gevoerd heeft. Ook had Dr KLOOS een belangrijk aandeel in het verdere verenigingsleven. Hij was gedurende de perioden van zijn penning-

meesterschap lid van de Commissie van Redactie van het Nederl. Kruidkundig Archief en van het *Recueil des Travaux botaniques néerlandais*. Van 1917 af was hij lid van de Commissie voor het Floristisch Onderzoek van Nederland; in 1932 werd hij wegens zijn grote verdiensten op dit gebied tot voorzitter van deze Commissie benoemd. In 1926 werd hij lid van de Commissie voor de Bibliotheek, het Herbarium en het Archief; bij het instellen van de Commissie voor de Bescherming der wilde Flora en van de Subcommissie voor het Zuiderzee-onderzoek werd hij ook als lid van deze commissies gekozen. Op 26 October 1945, ter gelegenheid van haar eeuwfeest, heeft onze Vereniging Dr KLOOS — met Dr JANSEN, Dr PULLE en Dr WACHTER —, tot erelid benoemd.

Meer nog dan door al deze diensten, die Dr KLOOS onze Vereniging bewees, gedenken wij hem om zijn bescheiden, hulpvaardig en beminnelijk karakter. Heeft hij niet vele van onze jongere floristen de weg gewezen tot het vak van hun keuze en heeft hij daarna niet een actief aandeel gehad in hun aller werk; door hen gezocht mede om zijn grote kennis en ervaring. Vele zijn dan ook de publicaties, die van zijn hand zijn verschenen in onze tijdschriften; de jaarlijkse lijst van aanwinsten en de kritische bewerkingen van een aantal moeilijke plantengeslachten, in het Nederl. Kruidkundig Archief verschenen, geven wel een goed beeld van zijn grote activiteit. Hoe groot het medeleven van Dr KLOOS in onze Vereniging was, blijkt ook daaruit, dat hij zijn bibliotheek aan onze Vereniging heeft nagelaten. In hem verloor het Bestuur en onze Vereniging wel haar trouwste lid.

4 Augustus overleed te Heemstede ons lid J.R.H. MULLER. Na aan de Universiteit van Amsterdam biologie gestudeerd te hebben, ging hij na het behalen van de Akte K IV over naar de Universiteit van Utrecht, waar hij na enkele jaren het doctoraal examen in de physische geografie aflegde. Intussen was hij reeds tot assistent in de systematische botanie te Amsterdam benoemd; zijn grote belangstelling ging uit naar de Mycologie. In hem verliezen wij een trouw bezoeker van onze bijeenkomsten.

Tot slot moet vermeld worden, dat op 10 November op 86-jarige leeftijd overleed ons corresponderend lid Dr O. APPEL, oud-president van de Biologische Reichsanstalt, een der meest vooraanstaande phytopathologen in Duitsland. Grote bekendheid verwierf hij door zijn vele publicaties op dit gebied. Vele malen heeft hij ons land bezocht.

Wij herdenken hen allen, daar zij, ieder naar eigen vermogen, het beste gegeven hebben voor de groei van de Botanische wetenschap en voor onze Vereniging, en omdat zij met zulke innige vriendschapsbanden aan ons verbonden waren.

Dit jaar kwam een grote reorganisatie in de structuur van onze Vereniging tot stand, nodig geworden door het besluit tot reorganisatie van onze tijdschriften, in het vorige verslagsjaar genomen. Het Bestuur heeft daarvan gebruik gemaakt door geheel nieuwe Statuten en een Huishoudelijk Reglement te ontwerpen, die na enkele wijzigingen door de ledenvergadering van 27 Januari werden goedgekeurd. Door de vele administratieve beslommingen, verbonden aan de wijziging van onze tijdschriften, werd het in druk verschijnen van deze nieuwe Statuten en van het Huishoudelijk Reglement, als ook het verschijnen van het nieuwe jaarboek, vertraagd. Zij kunnen thans echter binnen korte tijd tegemoet gezien worden.

Hoezeer het Bestuur ook betreurt, dat de financiële positie van de Vereniging het verder verschijnen van het Nederlandsch Kruidkundig Archief en van het Recueil des Travaux botaniques néerlandais niet toeliet, meent het toch, dat de reorganisatie, die inmiddels heeft plaats gevonden, in vele opzichten grote voordelen biedt. De Acta Botanica Neerlandica, tot welks uitgave al in 1951 besloten werd, verscheen voor het eerst in April 1952. Zowel de kwaliteit van deze uitgave als het uiterlijk zijn aanzienlijk verbeterd; de gepubliceerde artikelen hebben betrekking op zeer uiteenlopende onderwerpen; zij waren niet te lang, zodat de Acta voor velen aantrekkelijk moet zijn. De Redactie-Commissie komt regelmatig met een aantal Bestuursleden bijeen; de Commissie mag terecht tevreden zijn over het bereikte resultaat. Dit neemt echter niet weg, dat het Bestuur zich wel bewust is, dat nog tal van moeilijkheden overwonnen moeten worden en dat nog vele verbeteringen zullen moeten worden ingevoerd. Zij zal hiernaar met kracht blijven streven. Het Bestuur hoopt echter, dat vele leden van de nu geboden gelegenheid gebruik zullen maken om de waardevolle resultaten van hun wetenschappelijk werk in ons orgaan te publiceren. Alleen zó kan de Vereniging de door haar opgenomen taak blijven volbrengen.

Grote voldoening gaf het ook, dat bij de jaarswisseling het tweede deel van de Flora Neerlandica kon verschijnen. Het Bestuur streeft met grote kracht ernaar, dat ook de verdere delen nu spoedig kunnen volgen.

Ook dit jaar gaf de Vereniging blijk van grote activiteit. Vele waren de bijeenkomsten van de verschillende commissies, waarbij de leden belangrijke wetenschappelijke resultaten bekend maakten. Zeer duidelijk demonstreert zich hierin de grote bloei van onze Vereniging. Wel geeft het het Bestuur zorg, dat de activiteit zich meer en meer in de commissie-bijeenkomsten gaat voltrekken en minder in de algemene vergaderingen. Gestreefd wordt naar een goede verhouding in deze. Dit jaar werden 3 algemene vergaderingen gehouden. De jaarvergadering vond plaats op 27 Januari te Amsterdam; sprekers waren Dr C.G.G.J. VAN STEENIS en Mej. Dr Joh.C. WENT; eerstgenoemde sprak over *Rheophyten*; laatstgenoemde over *Boomziekten in de Verenigde Staten*. De tweede ledenvergadering werd op 17 Mei gehouden, eveneens in Amsterdam. Dr G. BREMER besprak *Het probleem van de vermeerdering van het chromosomenaantal bij soortsbastaardering van Saccharum in verband met de embryozakontwikkeling*, terwijl Dr H.C.D. DE WIT *Rumphius herdacht*. De derde bijeenkomst, tevens zomervergadering, vond plaats in het Laboratorium voor Bloembollenonderzoek te Lisse op 15 Juni. In deze zeer geslaagde en druk bezochte bijeenkomst hield onze Voorzitter, Dr E. VAN SLOGTEREN een inleiding over het werk in het laboratorium verricht, waarna enige van zijn medewerkers bepaalde problemen nader belichtten. Dr J.J. BEIJER sprak over het *Onderzoek betreffende de periodiciteit bij bloembollen*; Drs A.F.G. SLOOTWEG over *Wortelrot bij Narcissen* en Ir D.H.M. VAN SLOGTEREN over *Virusonderzoek en serologische diagnostiek*. In de middag werden vele fraaie demonstraties gegeven, betrekking hebbende op de onderzoeken, en werden de nieuwe uitbreidingen van het laboratorium bezichtigd. Men was zeer geboeid door de resultaten van het belangrijke en veelzijdige wetenschappelijk onderzoek, dat in het laboratorium te Lisse verricht wordt. Een woord van hartelijke dank moet ook hier gesproken worden voor de gastvrije ontvangst de Vereniging bij haar zomervergadering geboden.

Op de Jaarvergadering werd Dr S.J. VAN OOSTSTROOM als lid van het Bestuur gekozen. In de commissies werden zover als dit veroorloofd is de aftredende leden herkozen en in die voor welke de statuten een verplichte wijziging voorschrijven werden de door de commissies zelve voorgestelde nieuwe leden gekozen in de plaats van de aftredende.

Het ledental bedroeg aan het einde van het verslagjaar 462, waarvan 77 student-leden. Een aantal leden in Indonesië verblijvende of daar gewoonde hebbende, waarmee gedurende vele jaren geen contact meer verkregen kon worden, werden van de ledenlijst afgevoerd.

De financiën gaven als steeds grote zorgen, zodat vele voorgenomen projecten door gebrek aan middelen in hun uitvoering vertraagd moesten worden. De noodzakelijk geworden verhoging van de contributie werd op 1 Januari 1952 ingevoerd. Deze heeft praktisch geen achteruitgang in het ledental gegeven, zodat de verhoging ten volle aan de verwachtingen voldaan heeft. Grote dank zijn wij ook verschuldigd aan de Begunstigers en Donateurs, die onze Vereniging zo krachtig steunden. Vele problemen op financieel gebied konden onder de krachtige en voortvarende leiding van onze Voorzitter tot oplossing gebracht worden.

Zijn de financiële zorgen nog groot, de activiteit en bloei van de Vereniging is zelden zo groot geweest, zodat wij de toekomst vol vertrouwen tegemoet kunnen zien.

Amsterdam, 31 December 1952.

De eerste Secretaris,
A.W.H. VAN HERK

VERSLAG van de Commissie van Redactie voor de Acta Botanica Neerlandica over 1952

Nadat op 5 October 1951 de Koninklijke Nederlandse Botanische Vereniging een contract had aangegaan met de N.V. Noord-Hollandsche Uitgeversmaatschappij te Amsterdam inzake de uitgave van het nieuwe tijdschrift der Vereniging kon kort daarna worden begonnen met het drukken van het eerste nummer van Deel I. Dit verscheen op 31 Maart 1952, dus juist op het einde van het eerste kwartaal. Het bevat 156 bladzijden druks en 2 tweezijdig bedrukte buitentekstplaten. Er zijn 6 artikelen in opgenomen, waarvan er één als dissertatie is verspreid.

Ondanks het feit, dat de drukker steeds voldoende kopie had, verscheen het tweede nummer pas op 2 Augustus 1952, dus een maand te laat. Dit nummer telt 167 pagina's, 1 losse tabel en 3 buitentekstplaten, waarvan 1 tweezijdig bedrukt. Er zijn 11 artikelen in opgenomen, waarvan er één als dissertatie is gedistribueerd.

De derde aflevering verscheen op 1 December 1952, dus twee maanden na afloop van het derde kwartaal; een en ander als gevolg van het lang uitblijven van de drukproeven. Het bevat 164 bladzijden, verdeeld over 7 artikelen, waarvan er één als dissertatie is verspreid. Het nummer heeft 2 losse tabellen en 1 buitentekstplaat.

Inmiddels had de uitgever voldoende kopie ontvangen voor de vierde afle-

vering, waarvan de redactie op 2 Februari 1953 een opgemaakte proef ontving. Daar inmiddels de redactiecommissie had besloten, dat het deel zou worden voorzien van een index en deze pas kon worden samengesteld na het ontvangen van de laatste proef met definitieve paginering, moest de afwerking van het 1ste deel wachten, totdat de index zou zijn gezet. De proef daarvan werd op 28 Februari 1953 ontvangen.

De vierde aflevering zal, exclusief de index, 111 pagina's omvatten, verdeeld over 10 artikelen. Zij zal vermoedelijk in Maart 1953 verschijnen.

De uitgever heeft sedert einde Januari 1953 voldoende kopie voor de eerste aflevering van deel II.

De Commissie vergaderde in 1952 drie maal en wel op 30 Juni, 25 September en 17 December. In haar samenstelling kwam verandering door het overlijden van Dr Ir A.W. KLOOS. Hij werd opgevolgd door Ir J.L. VAN SOEST. Mej. Dr Joh. WESTERDIJK trad uit de Commissie en werd vervangen door Mej. Dr L.C.P. KERLING.

Utrecht/Haarlem,
1 Maart 1953.

De Secretarissen,
A.A. PULLE
W. VAN DIJK

VERSLAG van de Commissie voor de Bibliotheek, het Herbarium en het Archief over 1952

Het Herbarium is verrijkt met de verzameling van wijlen Dr BRAND, die deze aan de Vereniging legateerde; voorts schonk de secretaris 32 Taraxaca. Meer kwam niet in, zodat aan de leden wordt verzocht in komende jaren meer aandacht aan schenkingen voor het herbarium te willen wijden!

In 1952 zijn 783 herbariumnummers uitgeleend; aan het einde van het jaar bedroeg het totaal aantal uitleningen 2589 nummers.

Uit de Bibliotheek zijn in 1952 199 nummers uitgeleend. Aan boeken, overdrukken en brochures zijn 511 nummers binnengekomen, waaronder het legaat BRAND (116 nummers) en, voorzover reeds uitgezocht het legaat van wijlen KLOOS (182 nummers); voorts zijn 203 nummers door instituten en leden geschonken; van de laatste schonken meer dan 3 nummers: ARISZ (4), J.G. DE BRUYN (10), HOOGLAND (32), KERN (4), W. MEIJER (5), SIRKS (5), VAN SOEST (74).

In verband met de opheffing van het Recueil en van het Kruidkundig Archief, het verschijnen van de Acta Botanica, en in verband met de noodzakelijke bezuinigingen is het aantal ruilverbindingen in 1952 beperkt tot ruim tachtig. Aan tijdschriftdelen kwamen er dit jaar 641 afleveringen binnen.

's-Gravenhage,
18 Januari 1953.

De Secretaris,
J.L. VAN SOEST

VERSLAG van de Commissie voor het Floristisch Onderzoek over 1952

Dit jaar leed de Commissie een zwaar verlies door het overlijden van Dr Ir A.W. KLOOS. Sinds 1917 was hij lid der Commissie, sinds 1924 leider der excursies en sinds 1931 voorzitter. Zijn vele en belangrijke verdiensten op floristisch gebied zullen door ons niet vergeten worden.

Wederom werden een Paas- en Kerstvergadering te Amsterdam gehouden, die zich in een zeer talrijk bezoek mochten verheugen. De publicatie-mogelijkheden der daar gehouden voordrachten zijn helaas door het verdwijnen van Het Nederlandsch Kruidkundig Archief zeer beperkt.

De zomerexcursie der Commissie werd gehouden in de omgeving van Doetinchem en leverde onder leiding van REICHGELT interessante resultaten op.

Voorlopig werd het voorzitterschap waargenomen door ondergetekende en werd Th. REICHGELT als excursieleider aangewezen.

Amsterdam,
31 December 1952.

De Secretaris,
P. JANSEN

VERSLAG van de Commissie voor de Bescherming van de Wilde Flora over 1952

Wat onze Commissie over 1952 te rapporteren heeft, werd eigenlijk reeds in het voorgaande jaarverslag vermeld. Dat was het overlijden van den initiatiefnemer tot de oprichting ervan, onze onvergetelijke voorzitter Prof. Dr Th. WEEVERS.

Door zijn persoon zijn wij steeds in allerlei commissoriale werkzaamheden ingeschakeld geweest, zowel bij die welke de Contactcommissie voor Natuur- en Landschapsbescherming, als die welke officiële instanties moesten voorlichten.

Deze vertegenwoordiging hebben wij sinds zijn overlijden gemist. Wel zijn ons in het afgelopen jaar verschillende malen informaties gevraagd, maar actief beschermingswerk is door ons niet verricht.

Toch lijkt het blijven voortbestaan van de Commissie ons nuttig, al was het maar om voor onze Vereniging direct een orgaan gereed te hebben, dat kan optreden als een botanisch natuurbeschermingsbelang niet door een van de overkoepelende organisaties kan worden opgevangen. Dat zijn wij aan de nagedachtenis van Prof. WEEVERS verplicht.

Een tweede verlies leed onze Commissie nog door het overlijden van onze goede vriend Dr Ir A.W. KLOOS Jr, die — zij het eveneens met reglementair voorgeschreven onderbrekingen — van de oprichting af zitting had. Zijn ongeëvenaarde floristische kennis is ons altijd van grote steun geweest bij de juiste waardering van groeiplaatsen en terreinen, die onze aandacht vroegen. Ook hem zullen wij ten eerste missen.

De afzonderlijke leden van onze Commissie, van wie verscheidene ook zitting hebben in diverse andere natuurbeschermingsorganen, kunnen bij hun actie ten behoeve van botanisch belangrijke objecten zich gesteund voelen, en

ook inderdaad aan gezag winnen, doordat zij onze Commissie en daardoor de Kon. Nederl. Botanische Vereniging achter zich weten.

Bergen op Zoom,
31 December 1952.

De Secretaris,
Jan G. SLOFF

VERSLAG van de Commissie voor de Phytopathologie over 1952

In de vacature, ontstaan door het periodiek aftreden van Dr J.W.M. ROODENBURG werd voorzien door de benoeming van Dr K. HARTSUYKER. Het aantal contribuanten bedraagt thans 34.

Er werden dit jaar twee vergaderingen gehouden. De eerste op Vrijdag 2 Mei te Utrecht over *Bodemparasieten en bodemviren*. De sprekers waren: Dr A.J.P. OORT, *Inleiding*; Dr L.C.P. KERLING, *Fusarium oxysporum pisi, de veroorzaker van de Amerikaanse vaatziekte van de erwit*; Ir A.P. KOLE, *Spongospora subterranea, de veroorzaker van de poederschurft bij de aardappel*; A.F.G. SLOOTWEG, *Over de oorzaak van het wortelrot van de narcis*; Dr Ir M. COSTENBRINK, *Heterodera rostochiensis, de veroorzaker van de aardappelmoeheid*; Ir J.P.J. VAN DER WANT, *Bodemviren*. De tweede vergadering werd op Zaterdag 22 November te Utrecht gehouden. De agenda vermeldde als sprekers: Dr A.J.P. OORT, *Taksterfte in bramen*; Dr D. MULDER, *Virusziekten in fruitgewassen*; Ir A.B.M. BEEMSTER, *Virusonderzoek bij stoppelknollen*.

Lisse,
7 Januari 1953.

De Secretaris,
A.F.G. SLOOTWEG.

VERSLAG van de Commissie voor de Biosociologie en het Veenonderzoek van Nederland over 1952

In de samenstelling van de commissie ontstonden enige wijzigingen. Dr J. HEIMANS trad als lid en voorzitter af; in zijn plaats werd het commissielid Dr J. LANJOUW tot voorzitter benoemd. Als commissielid werd Dr J. HEIMANS door Dr F. FLORSCHÜTZ vervangen. Ook de heer T. VAN DER HAMMEN trad af in verband met zijn vertrek naar het buitenland; in deze vacature werd niet voorzien.

Ook in dit verslagjaar organiseerde de commissie weer twee bijeenkomsten: de 29ste en 30ste Nederlandse Dag voor Biosociologie en Palaeobotanie, resp. op Donderdag 17 April en Vrijdag 19 December, beide in de collegezaal van het Botanisch Laboratorium te Utrecht. Op verzoek van verschillende leden werden deze beide dagen bij wijze van proef op werkdagen gehouden. Gezien de opkomsten kan dit experiment als geslaagd worden beschouwd. De 29ste Dag werd door 32 leden en 11 introducés bijgewoond. Voor de middagpauze spraken de heer H.J.W. SCHIMMEL uit Utrecht over *De vegetatie van het Zwin*; Ir W.G. BEEFTINK uit Wageningen over *Vegetatie en standplaatsfactoren van*

de schorren en duinen van de Kaloot en Dr D.M. DE VRIES eveneens uit Wageningen, mede namens Ir Th.A. DE BOER, over *Oecologische indicatie in cijfers*. Na de middagpauze, tijdens welke de lunch werd gebruikt in het Universiteitshuis, was eerst het woord aan de heer J. HENDRIKS uit Amsterdam, die mede namens de heer B. BELDEROK sprak over *Palynologie rondom Amsterdam*. Vervolgens hield de heer H. DOING KRAFT uit Wageningen een voordracht over *De bosvegetatie van de binnenduinrand van Den Haag tot Bergen*. Tenslotte voerde Ir K.J. ZANDSTRA uit Wageningen het woord over *Uitkomsten van een onderzoek naar de akkeronkruidvegetatie van de Wageningse Eng*.

De 30ste Dag werd bezocht door 33 leden en 13 introducés. Voor de middagpauze spraken Ir D. BURGER uit Wageningen en Dr M.F. MÖRZER BRUYS uit Utrecht over resp. *Bodemkundige onderzoeken in het ongestoorde en bewerkte profiel van het droge Querceto-Betuletum* en *Een biosociologisch onderzoek van een vochtige duinvallei in het buitenduin van Terschelling*. Nadat de lunch gebruikt was in het Universiteitshuis spraken achtereenvolgens de heren Chr.G. VAN LEEUWEN (Utrecht) over *Een biosociologisch onderzoek van een binnenduinpan op Terschelling*, W.H. DAMMAN (Wageningen), mede namens Dr D.M. DE VRIES, over *Toetsing van associaties aan soortencombinaties* en Dr V. WESTHOFF (Wageningen) over *De plantensociologische tuin in Hannover*.

Ens (N.O.polder),
Januari 1953.

De Secretaris,
D. BAKKER

VERSLAG van de Commissie voor de Plantenphysiologie over 1952

In de jaarvergadering van 27 Januari 1952 werd in de vacature, ontstaan door het periodiek aftreden van het lid der commissie Mej. Dr A.M. HARTSEMA te Wageningen, voorzien door de benoeming van Dr P.A. ROELOFSEN tot lid van de commissie.

In verband met de grote belangstelling en de talrijke zich aanmeldende sprekers werd dit jaar voor het eerst een dag-vergadering door onze commissie belegd. Op deze dag, die gehouden werd Zaterdag 3 Mei in het Botanisch Laboratorium te Utrecht werden de volgende voordrachten gehouden: Dr J.B. THOMAS (Utrecht): *Het recente onderzoek van de biophysische werkgroep te Utrecht*; Ir J.L.P. VAN OORSCHOT (Wageningen): *Over het rendement van de lichtenergie bij de groei van Chlorella en van hogere planten*; Dr B.J.D. MEEUSE (Delft): *Een bijzondere amylase van schimmels; Rietsuikervorming bij planten*; R.J. HELDER (Groningen): *Het verband tussen de fosfaatopname door maisplanten en de concentratie van het milieu*; P. DULLEMAYER (Leiden): *De invloed van de temperatuur op de snelheid van het transport van organische stoffen bij luchtwortels van Cissus sicyoides* en Dr Th. VAN DEN HONERT (Leiden): *Wetmatigheden bij de ionenopname*. Deze vergadering werd bijgewoond door 58 leden en 2 introducés.

Op Zaterdag 13 December werd, eveneens in het Botanisch Laboratorium te Utrecht een middagvergadering belegd, waarop de volgende voordrachten gehouden werden: Mej. Dr J.C. SOBELS (Utrecht): *Over de betekenis van mi-*

neralen en koolhydraten voor in reïncultuur gekweekte Myxomyceten; Dr T.H. VAN DEN HONERT (Leiden): *Diffusieweerstanden bij de opname van ammonium door aardappelschijfjes*; Mej. W. TERPSTRA (Utrecht): *Over de aard van de groeistof in het Avenacoleoptiel* en H. KETELLAPPER (Utrecht): *De invloed van heteroauxine op de elastische eigenschappen van de celwand*. Deze vergadering werd bijgewoond door 43 leden en 3 introducés.

Amsterdam,
1 Januari 1953.

De Secretaris,
A. QUISPÉL

VERSLAG van de Commissie voor de Plantensystematiek en Plantengeografie over 1952

In de samenstelling van de commissie ontstond een wijziging door het aftreden van de voorzitter, Dr J. LANJOUW. In zijn plaats werd als lid van de commissie gekozen Dr C.E.B. BREMEKAMP, terwijl het commissielid Dr S.J. VAN OOSTSTROOM door de commissie aangewezen werd als voorzitter.

De commissie organiseerde in het verslagjaar twee vergaderingen, resp. op Zaterdagmiddag 5 April en Zaterdagmiddag 8 November; beide vergaderingen werden gehouden in het Botanisch Laboratorium van de Rijksuniversiteit te Utrecht. Op de eerste vergadering spraken achtereenvolgens Dr P. VERMEULEN over *De Amerikaanse soorten van het genus Orchis en hun systematische positie*; Dr R.C. BAKHUIZEN v.d. BRINK over *Bijzonderheden over de Malesische Rubiaceae*, Dr P. VAN ROYEN over *Achras en Manilkara* en P.A. FLORSCHÜTZ over *De vegetatie van een „rotssavanne” in Suriname*. Korte mededelingen deden Mejuffrouw Dr J. Th. KOSTER over *Zeldzame Nederlandse zee-wieren*; Dr C.G.G.J. VAN STEENIS over *Nieuw-Guinese Nothofagus* en Dr R.A. MAAS GEESTERANUS over *Nederlandse Physcia's*. Deze vergadering werd bijgewoond door 33 leden.

Op de tweede vergadering werd gesproken door Mejuffrouw Dr Ch. H. ANDREAS over *De oude geschiedenis van de Groningse Hortus*; Dr C.E.B. BREMEKAMP over *Het systeem van Caesalpini*; J.P. SCHULZ over *De betekenis van de anatomie voor de systematiek van de houtige Zuid-Amerikaanse Apocynaceae* en Dr F.P. JONKER over *De Araceae van Suriname*. Tijdens deze vergadering deed Dr H. J. VENEMA een korte mededeling over *Monstrueuze strobili van Equisetum arvense*. In zijn openingswoord tot deze vergadering, die door 32 personen werd bijgewoond, sprak de voorzitter een speciaal woord van welkom tot een buitenlandse gast, Prof. P.G. JORDAAN uit Stellenbosch, Zuid-Afrika.

Utrecht,
1 Januari 1953.

De Secretaris,
F.P. JONKER

**VERSLAG van de Commissie tot het nazien van Rekening en
Verantwoording en de bescheiden van de Penningmeester over 1952**

Daartoe aangewezen in de Algemene Vergadering van 1 Februari 1953 hebben ondergetekenden op 23 Februari 1953 de boeken van de penningmeester gecontroleerd en deze volmaakt in orde bevonden. Zij verlenen dan ook volgaarne het advies hem te dechargeren.

Leiden,
23 Februari 1953.

S.E. DE JONGH.
W.K.H. KARSTENS.

Verlies- en Winstrekening over 1952

Kok-Ankersmit-Fonds

Uitgaven	fl 1.005,70	Ontvangsten	fl 1.320,45
Crediteuren	fl 600,--	Koersverschil	fl 595,01
Saldo winst	fl 309,76		
	fl 1.915,46		fl 1.915,46

Brumund-Fonds

Uitgaven	fl 1.000,--	Ontvangsten	fl 600,63
Saldo winst	fl 1.034,53	Koersverschil	fl 1.433,90
	fl 2.034,53		fl 2.034,53

Vrije Fonds

Lidmaatschappen	fl 554,05	Interest	fl 743,14
Herbarium en Bibliotheek	fl 650,90	Koersverschil	fl 360,41
Vergaderingen	fl 585,13	Contributies	fl 2.347,--
Onkosten	fl 297,47	Debiteuren	fl 3.824,74
Oninbare contributies (Indonesische leden)	fl 1.577,50	Nederl. Kruidkundig Archief	fl 499,35
Saldo winst	fl 6.734,58	Recueil des Trav. Bot. Neerl.	fl 1.028,35
		Flora Neerlandica	fl 1.595,89
		Zuiderzee Commissie	fl -,75
	fl 10.399,63		fl 10.399,63

BALANS, 1 Januari 1953

Kok-Ankersmit-Fonds

Effecten	fl 35.260,71	Saldo op 1 Jan. 1952	fl 36.818,87
Kassaldo	fl 2.467,92	Saldo winst	fl 309,76
		Crediteuren	fl 600,--
	-----		-----
	fl 37.728,63	Fonds op 1 Jan. 1953	fl 37.728,63

Brumund-Fonds

Effecten	fl 18.438,05	Saldo op 1 Jan. 1952	fl 25.171,52
Kassaldo	fl 7.768,--	Saldo winst	fl 1.034,53
	-----		-----
	fl 26.206,05	Fonds op 1 Jan. 1953	fl 26.206,05

Vrije Fonds

Effecten	fl 5.039,41	Saldo op 1 Jan. 1952	fl 11.143,31
Kassaldo	fl 12.455,07	Saldo winst	fl 6.734,58
Te innen interest	fl 518,--	Crediteuren (Herbarium	
Te innen contributies	fl 1.394,--	en Bibl.)	fl 243,80
Te innen Acta (Rijks-		Crediteuren (diversen)	fl 8.080,--
bijdr.)	fl 1.676,40		
Overige debiteuren	fl 5.118,81		
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	fl 26.201,69		fl 26.201,69

BEGROTING 1953

Kok-Ankersmit-Fonds

Inkomsten	fl 1.100,--	Onkosten	fl 20,--
		Bibliotheek en Herbarium	fl 300,--
		Flora Neerlandica	fl 625,--
		Natuurmonumenten	fl 50,--
		Wahlenbergia-terrein	fl 55,--
		Stichting Wetensch. Duin-	
		onderz.	fl 50,--
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	fl 1.100,--		fl 1.100,--

Brumund-Fonds

Inkomsten	fl 850,--	Onkosten	fl 20,--
		Flora Neerlandica	fl 605,--
		Commissie v. Floristiek	fl 25,--
		Commissie v. Zuiderzee-onderz.	fl 25,--
		Commissie v. Bescherming wilde flora	fl 25,--
		Commissie v. Biosociologie	fl 25,--
		Bibliotheek en Herbarium	fl 100,--
		Instituut Vegetatie-onderz. Nederl.	fl 25,--
	fl 850,--		fl 850,--

Vrije Fonds

Inkomsten	fl 300,--	Onkosten	fl 500,--
Contributies	fl 6.100,--	Vergaderingen	fl 350,--
Verkoop	fl 1.400,--	Bibliotheek en Herbarium	fl 400,--
		Commissie v. Physiologie	fl 25,--
		Commissie v. Phytopathologie	fl 25,--
		Zoölogisch Station Den Helder	fl 100,--
		Plantenziektenk. Ver.	fl 10,--
		Biologische Raad	fl 10,--
		Contactcommissie	fl 50,--
		Ned. Natuur en Geneesk. Congres	fl 4,--
		Int. Natuur-Bescherming	fl 5,--
		Acta Botanica Neerlandica	fl 5.500,--
		Flora Neerlandica	fl 770,--
		Centr. Bureau v. Schimmelcult.	fl p.m.
		Onvoorzien	fl 51,--
	fl 7.800,--		fl 7.800,--

NOTULEN OVER HET JAAR 1952

JAARVERGADERING gehouden op Zondag 27 Januari 1952 in het
HUGO DE VRIES-Laboratorium te Amsterdam

Aanwezig waren 39 leden: de presentielijst vermeldt de namen van de Dames en Heren AGSTERIBBE, VAN ASPEREN DE BOER, A.J. BIANCHI, BOTTELIER, BREMEKAMP, VAN DIJK, FRAHM-LELIVELD, A. GORTER, v. HATTUM, HEIMANS, VAN HERK, HEYBROEK, JONKER, KARSTENS, KERLING, KLOOS, KONINGSBERGER, v.d. LAAN, LANJOUW, DE LEEUW, J.C. LINDEMAN, A. MENNEGA, VAN OOSTSTROOM, PULLE, VAN ROYEN, VAN SOEST, SLOFF, VAN SLOGTEREN, STOMPS, TAMMES, THOMAS, VERMEULEN, WESTERDIJK, R.J. DE WIT, ZEYLEMAKER, ZWILLENBERG; 3 namen waren niet leesbaar. Van het Bestuur was Mej. Dr A. JAARVELD wegens ziekte verhinderd.

De Voorzitter opent de vergadering en herdenkt de overleden leden Prof. Dr Th. WEEVERS en Dr F.J.M. OFFERIJNS. In een warm betoog schetst hij de vele verdiensten van WEEVERS, in het bijzonder voor onze Vereniging. Steeds heeft WEEVERS zijn beste krachten aan de Vereniging gegeven; vele malen heeft het Bestuur een beroep op hem moeten doen om over moeilijke problemen van advies te dienen. Wegens zijn grote verdiensten voor de Vereniging werd hem in 1940 het erelidmaatschap aangeboden. Tot kort voor zijn overlijden nam hij nog actief deel aan het werk van vele commissies. Na een kortdurende ziekte overleed hij 15 Januari 1952. Het Bestuur was bij de begrafenis tegenwoordig. Dr F.J.M. OFFERIJNS heeft vele jaren een actief aandeel gehad in het werk van onze Vereniging; een aantal belangwekkende voordrachten heeft hij in onze bijeenkomsten gehouden. Na een zeer langdurig lijden overleed hij 8 Januari 1952; hij werd in alle stilte ter aarde besteld. Voorts herdacht de Voorzitter het overlijden van drie Biologen: C.J. KONINGSBERGER, DAMMERMAN en JANSONIUS; zij waren vele jaren lid van onze Vereniging. Gezien de grote betekenis, die zij op het verloop van de ontwikkeling van de Biologie in ons land gehad hebben, is een korte herdenking van hun overlijden ook in onze Vereniging op zijn plaats. In enige ogenblikken van stilte herdenken de aanwezigen de overledenen.

Vervolgens worden de notulen van de vorige bijeenkomst gelezen en goedgekeurd.

Daarna brengt de 1ste Secretaris zijn verslag uit over de lotgevallen van de Vereniging in het jaar 1951. Dit wordt, na het herstellen van een kleine onnauwkeurigheid goedgekeurd. Ook de verslagen van de Permanente Commissies worden voorgelezen en aanvaard. Zij zullen t.z.t. in het Jaarboek van de Vereniging worden opgenomen.

De Penningmeester legt nu verantwoording af van het door hem in 1951 gevoerde beheer. Het blijkt, dat de Vereniging een saldo-verlies heeft geleden van fl 3.955,47. Dit ondanks het feit, dat in 1951 geen nieuw deel van de tijd-

schriften is verschenen. Het saldo-verlies is ontstaan door de sterk verhoogde kosten, verbonden aan het uitgeven van de tijdschriften en de *Flora Neerlandica*.

Het *Kok-Ankersmit-Fonds* staat thans te boek voor fl 36.818,87 (oorspronkelijk fl 30.000,--) en het *Brumund-Fonds* voor fl 25,171,52 (oorspronkelijk fl 25.000,--). De voorzitter wijst er op, dat deze bedragen nominaal zijn genomen, maar dat door de waardevermindering van het geld een reële afname van het inkomen der Vereniging uit deze fondsen is ontstaan. Men hoopt voor het voortzetten der publicaties subsidies te kunnen verkrijgen.

Na een korte discussie over de vraag over de grootte van het niet-belegde bedrag en de samenstelling van het effecten-bezit der Vereniging, worden de rekening en verantwoording goedgekeurd.

Daarna dient de Penningmeester de begroting voor het jaar 1952 in. Deze begroting geeft weinig aanleiding tot discussie. Mevr. WESTERDIJK merkt op, dat zij altijd zeer dankbaar geweest is voor de hulp, die de Vereniging aan haar laboratorium heeft gegeven. Nu de finantiële toestand van de Vereniging zeer zorgelijk is, begrijpt zij, dat het subsidie van de begroting moet worden afgevoerd. Zij zou echter gaarne een band met de Vereniging willen houden. Besloten wordt daarop het subsidie aan het Laboratorium pro memori op de begroting te plaatsen.

Voor het uitgeven van de *Acta Botanica Neerlandica* wordt geraamd fl 12.000,--, terwijl voor de *Flora Neerlandica* fl 850,-- beschikbaar gesteld kan worden. Indien echter een verder deel van de *Flora* dit jaar nog gereed zou komen, kan dit verschijnen en zal het hiervoor benodigde bedrag aan het *Kok-Ankersmit-Fonds* of het *Brumund-Fonds* onttrokken kunnen worden. De begroting wordt daarna aangenomen.

Een discussie wordt gevoerd naar aanleiding van de mededelingen van de Voorzitter over de *Acta Botanica Neerlandica*. Het Bestuur wil trachten dit tijdschrift een zo gevarieerd mogelijke inhoud te geven door vele, vooral korte artikelen te plaatsen over zeer uiteenlopende onderwerpen. De Voorzitter wekt alle leden op hieraan volledig mede te willen werken. In het bijzonder zou hij gaarne zien, dat ook van Phytopathologische zijde bijdragen werden ingezonden. Verder heeft de Voorzitter een vrij groot bedrag ontvangen van Donateurs en Begunstigers voor het uitgeven van de *Acta* en konden ook enkele subsidies van laboratoria worden verhoogd. Getracht wordt nog meer subsidies te krijgen, ook van de Regering. Dank zij de reeds van vele zijden ontvangen steun en medewerking kan de *Acta* nu verschijnen en mag men met vertrouwen de verdere ontwikkeling van het tijdschrift tegemoet zien. Dr KONINGSBERGER dankt de Voorzitter namens de Vergadering voor de vele moeite, die hij zich gegeven heeft om dit gunstige resultaat te bereiken.

Dr HEIMANS deelt nog mede, dat de Firma JUNK sole-agent voor de *Flora Neerlandica* is geworden. Hij heeft in zijn catalogus flink de aandacht gevestigd op de *Flora*, en een speciaal inlegselsel laten maken. Ook in het buitenland zal het onder de aandacht van de botanici gebracht worden.

Naar aanleiding van een vraag over het *Wahlenbergia*-terrein deelt de Penningmeester mede, dat hierop toezicht wordt uitgeoefend. De leden JONKER en VAN OOSTSTROOM zullen het terrein blijven controleren.

Op voorstel van het Bestuur benoemt de Vergadering tot leden van de Commissie tot het nazien van rekening en verantwoording van de Penningmeester

de Heren Dr C.E.B. BREMEKAMP en Dr F.P. JONKER. Beide leden aanvaarden deze benoeming.

Bij acclamatie worden de per convocatie voorgestelde personen tot lid van de Kon. Nederl. Botanische Vereniging benoemd; eveneens een aantal andere, die met dispensatie van artikel 6, 1ste lid, der Statuten, staande de vergadering zijn voorgesteld.

De Voorstellen van de Permanente Commissies tot het benoemen van de nieuwe leden voor deze Commissies worden bij acclamatie aanvaard; dit zijn: Commissie voor Bibliotheek, Herbarium en het Archief: Dr Ir A.W. KLOOS (herkozen).

Commissie voor Floristisch Onderzoek van Nederland: J.G. SLOFF (herkozen). Subcommissie voor het Zuiderzee-Onderzoek: Mej. Dr K.J. HOCKE HOOGENBOOM (herkozen).

Commissie voor de Bescherming van de Wilde Flora: Dr Ir A.W. KLOOS.

Commissie voor de Phytopathologie: Dr K. HARTSUIJKER.

Commissie voor de Biosociologie en het Veenonderzoek van Nederland: Mr Dr F. FLORSCHUTZ.

Commissie voor de Plantenphysiologie: Dr P.A. ROELOFSEN.

Commissie voor de Plantensystematiek en Plantengeografie: Dr C.E.B. BREMEKAMP.

Over de benoeming van de leden van de Permanente Commissie voor de Acta Botanica Neerlandica zullen nadere voorstellen van het Bestuur volgen, zodra het Reglement van deze Commissie is vastgesteld.

Volgens de Statuten treedt in deze vergadering Dr J. LANJOUW af als voorzitter. Het Bestuur heeft het volgende voorstel ingediend om in deze vacature te voorzien: 1. Dr S.J. VAN OOSTSTROOM; 2. Dr F.P. JONKER. Geen der leden heeft van het recht om tegencandidaten voor te stellen, gebruik gemaakt. Bij de nu gehouden schriftelijke stemming worden op Dr VAN OOSTSTROOM 31 geldige stemmen uitgebracht: op Dr JONKER 5; twee stemmen waren blanco en één was van onwaarde. Daarmee is Dr S.J. VAN OOSTSTROOM als lid van het Bestuur gekozen. Desgevraagd door de Voorzitter, verklaart Dr VAN OOSTSTROOM de benoeming te willen aanvaarden.

De Voorzitter dankt het aftredende lid, Dr LANJOUW, voor het vele werk, dat hij in het Bestuur voor de Vereniging heeft verricht en voor de zeer prettige en hartelijke wijze, waarop de samenwerking steeds heeft plaats gevonden.

Aan de orde is het Voorstel van het Bestuur tot wijziging van de Statuten en tot het aanvaarden van een Huishoudelijk Reglement. Deze wijzigingen zijn noodzakelijk geworden door de veranderingen, die het uitgeven van de Acta Botanica Neerlandica heeft meegebracht.

Ingekomen is een schrijven van Dr BREMEKAMP, waarin hij een aantal wijzigingen op het voorstel van het Bestuur aanbeveelt. Het Bestuur is Dr BREMEKAMP zeer erkentelijk voor de grondige wijze, waarop hij de voorstellen bestudeerd heeft; het zal gaarne de meeste van deze wijzigingen overnemen.

Een voorstel van Dr WELLENSIEK om het student-lidmaatschap nog tot twee jaar na het doctoraal-examen te laten voortduren, wordt niet aanvaard. Leden, die bij de betaling van hun contributie moeilijkheden ondervinden, kunnen de-

ze steeds aan het Bestuur voorleggen. Dit is gerechtigd zo nodig vermindering van contributie te verlenen. Op een vraag van Dr BOTTELIER of een student-lid, die in December zijn doctoraal-examen aflegt, direct met 1 Januari d.a.v. als gewoon lid wordt ingeschreven, antwoordt de Voorzitter, dat ook deze kwestie in vertrouwen aan het Bestuur kan worden overgelaten.

Een discussie ontstaat over de redactie van art. 2 van de Statuten. Hier wordt de vraag gesteld, of het niet beter is Indonesië te vervangen door Malesië en of het niet gewenst is, hangende de besprekingen van de Ronde Tafel Conferentie, de aanduiding Nederlandse Antillen te wijzigen. Verschillende leden geven hierover hun mening. Besloten wordt de redactie aldus op te stellen: „.....de kennis der Flora van Nederland en die gebieden waar van oudsher Nederlandse botanici werkzaam zijn of geweest zijn”.

De gewijzigde voorstellen door het Bestuur ter vergadering ingediend worden daarop aangenomen.

Deze wijzigingen betreffen de volgende punten:

- a. de naam van de Vereniging, de Statuten en het Huishoudelijk Reglement worden in de nu geldende spelling geschreven;
- b. wijziging van de Statuten zijn:

1. Algemene Bepalingen

Artikel 1

De Vereniging draagt de naam: „KONINKLIJKE NEDERLANDSE BOTANISCHE VERENIGING”.

Zij is gevestigd te Amsterdam.

Artikel 2

De Vereniging stelt zich ten doel de bevordering van de Botanie in haar gehele omvang, in het bijzonder ook de bevordering van de kennis der flora van Nederland en die gebieden waar van oudsher Nederlandse botanici werkzaam zijn of geweest zijn.

Artikel 3

De middelen ter bereiking van dit doel zijn in hoofdzaak de volgende:

- a. het houden van vergaderingen,
- b. het maken van excursies,
- c. het bijeenbrengen van een herbarium en verdere verzamelingen,
- d. het bijeenbrengen van een boekerij,
- e. het uitgeven van verschillende werken,
- f. het ondersteunen van onderzoekingen of uitgaven.

Artikel 4

Het verenigingsjaar loopt van 1 Januari tot en met 31 December.

II. Leden, Contribuanten, Donateurs en Begunstigers

Artikel 5

De Vereniging heeft ereleden, corresponderende leden, leden en student-leden.

Artikel 6

De leden worden op een vergadering, bij volstrekte meerderheid van de stemmen der aanwezige leden, benoemd. Daartoe moeten zij voorgesteld zijn door minstens drie leden; hun namen, evenals die der voorstellers, moeten op het convocatiebillet van de vergadering worden vermeld.

Student-leden kunnen worden zij, die een studie, waarvan de botanie een onderdeel vormt, aan een Universiteit of Hogeschool hebben aangevangen, maar nog niet door een doctoraal-examen of ingenieursexamen hebben volbracht. Zij worden op een ledenvergadering benoemd. Daartoe moeten zij voorgesteld zijn door minstens drie leden; hun namen, evenals die der voorstellers, moeten op het convocatiebillet van de vergadering worden vermeld. Studenten-leden, die hun studie aan een Universiteit of Hogeschool hebben volbracht of afgebroken, worden met ingang van het eerstvolgende verenigingsjaar als leden ingeschreven.

Alle in dit artikel genoemde leden hebben stemrecht. Het bedrag van de jaarlijkse contributie en die van de daarop toegestane verminderingen worden bij Huishoudelijk Reglement vastgesteld.

Artikel 7

Corresponderende leden zijn verdienstelijke buitenlanders, die met de Vereniging in verbinding willen staan, en die als zodanig benoemd worden op dezelfde wijze als de gewone leden, met dien verstande, dat zij door minstens 10 leden moeten zijn voorgesteld. Zij brengen een adviserende stem uit en betalen geen contributie.

Artikel 8

Ereleden zijn zij, die op voorstel van het Bestuur, of van minstens 20 leden, met minstens $\frac{2}{3}$ van de stemmen der aanwezige leden, op een ledenvergadering benoemd worden. Zij hebben stemrecht, maar betalen geen contributie.

Artikel 9

Alleen leden, student-leden en ereleden der Vereniging zijn bevoegd tot het doen van alle voorstellen, welke zij in het belang der Vereniging wenselijk achten.

Over zodanige voorstellen wordt door de ledenvergadering beslist.

Artikel 10

Personen, die geen lid van de Vereniging zijn, kunnen als contribuant worden toegelaten. Zij verkrijgen het recht de vergaderingen van een der Commissies, bedoeld in art. 17 bij te wonen en ontvangen de convocatiebilletten van

deze Commissie. Zij betalen een jaarlijkse bijdrage, vast te stellen door het Bestuur.

Zij hebben geen stemrecht en ontvangen geen uitgaven der Vereniging.

Bij Huishoudelijk Reglement wordt vastgesteld voor welke Commissies contribuanten kunnen worden toegelaten. Het Bestuur beoordeelt, welke personen als contribuant worden toegelaten.

Artikel 11

Als donateur kunnen worden toegelaten alle natuurlijke of rechtspersonen, die tot steun van het doel der Vereniging jaarlijks een door henzelf te bepalen bedrag, waarvan de grenzen bij Huishoudelijk Reglement worden vastgesteld, in de kas van de Vereniging storten. Zij geven daartoe het verlangen te kennen aan het Bestuur, dat over de toelating beslist.

Donateurs hebben het recht de vergaderingen bij te wonen; zij hebben geen stemrecht.

Artikel 12

Als begunstiger kunnen worden toegelaten alle natuurlijke of rechtspersonen, die tot steun van het doel van de Vereniging jaarlijks een door henzelf te bepalen bedrag in de kas van de Vereniging storten; dit bedrag moet echter hoger zijn dan het maximum bedrag, dat bij Huishoudelijk Reglement voor donateurs is vastgesteld. Zij geven daartoe het verlangen te kennen aan het Bestuur, dat over de toelating beslist.

Begunstigers hebben het recht de vergaderingen bij te wonen; zij hebben geen stemrecht.

Artikel 13

Het lidmaatschap eindigt door schriftelijke opzegging, bij overlijden of door vervallen-verklaring door de Ledenvergadering. Voor de vervallen-verklaring is minstens 2/3 van de stemmen der aanwezige leden vereist.

De opzegging van het lidmaatschap moet geschieden aan het adres van de eerste secretaris voor 1 December van het lopende jaar en wordt dan van kracht met ingang van het eerstvolgende verenigingsjaar.

Het in dit artikel gestelde geldt ook voor contribuanten, donateurs en begunstigers.

III. Bestuur en Permanente Commissies

Artikel 14. Ongewijzigd art. 11.

Artikel 15. Ongewijzigd art. 12 (59 veranderen in 25).

Artikel 16. Ongewijzigd art. 13.

Artikel 17

De ledenvergadering kan Permanente Commissies instellen, wier taak en bevoegdheden bij Huishoudelijk Reglement worden omschreven. De leden van

deze Permanente Commissies worden door en uit de in art. 6 en 8 genoemde leden gekozen op een wijze en voor een tijdvak bij Huishoudelijk Reglement te bepalen.

Artikel 18

Elke Permanente Commissie kan door de ledenvergadering worden ontbonden. In zodanig geval moet in de volgende ledenvergadering een nieuwe Commissie worden gekozen. Ook kan de ledenvergadering een Permanente Commissie opheffen.

IV. Geldmiddelen

Artikel 19. Ongewijzigd art. 41.

Artikel 20. Ongewijzigd art. 42.

Artikel 21. Ongewijzigd art. 43.

Artikel 22. Ongewijzigd art. 44.

Artikel 23

De inkomsten van de Vereniging bestaan uit:

- a. de inkomsten uit het kapitaal der Vereniging,
- b. bijdragen der gewone leden, student-leden, contribuanten, donateurs en begunstigers,
- c. subsidies van andere Verenigingen of Instellingen,
- d. opbrengst van uitgaven der Vereniging,
- e. verdere inkomsten van verschillende aard.

Het bedrag der contributies van de leden wordt bij Huishoudelijk Reglement vastgesteld.

Artikel 24

De Penningmeester beheert, onder zijn persoonlijke verantwoordelijkheid, met inachtneming van de in de Statuten en in het Huishoudelijk Reglement ter zake geldende bepalingen, de geldmiddelen der Vereniging.

V. Ledenvergaderingen

Artikel 25.

Jaarlijks wordt minstens één ledenvergadering gehouden, zoveel mogelijk in Januari, welke in de eerste plaats bestemd is voor de afdoening van huishoudelijke zaken. Deze vergadering wordt in andere artikelen van de Statuten en in het Huishoudelijk Reglement aangeduid als jaarvergadering.

Artikel 26

Het Bestuur brengt in de jaarvergadering een door de eerste secretaris ont-

worpen verslag uit omtrent de handelingen en bevindingen der Vereniging in het afgelopen jaar. Tevens legt het de ontwerp-verslagen van de Permanente Commissies over. De vergadering stelt de verslagen vast.

Het Bestuur legt verder de rekening van de Penningmeester en de verantwoording van zijn beheer over het afgelopen jaar over. Deze rekening moet op een in het Huishoudelijk Reglement te omschrijven wijze worden nagezien en goedgekeurd.

Tevens dient het Bestuur een door de Penningmeester ontworpen en in een Bestuursvergadering voorlopig vastgestelde begroting der inkomsten en uitgaven voor het nieuwe jaar in. In het Huishoudelijk Reglement wordt nader omschreven, welke posten op de begroting afzonderlijk vermeld moeten staan. De jaarvergadering stelt de begroting vast.

Artikel 27. Ongewijzigd art. 60.

Artikel 28. Ongewijzigd art. 62.

Artikel 29

Vergaderingen, door een Permanente Commissie bijeengeroepen, zijn geen ledenvergaderingen in de zin van deze Statuten.

Artikel 30

Voor zover in deze Statuten niet anders is bepaald, is voor het nemen van een besluit de volstrekte meerderheid der geldige stemmen voldoende. Blanco stemmen zijn van onwaarde.

Artikel 31

Al hetgeen verder op de vergaderingen betrekking heeft, wordt geregeld bij Huishoudelijk Reglement.

VI. Huishoudelijk Reglement

Artikel 32

Tot regeling van een aantal punten, die in deze Statuten daarvoor aangewezen zijn, alsmede van enkele andere aangelegenheden, wordt door een voor dit doel belegde ledenvergadering een Huishoudelijk Reglement vastgesteld. De bepalingen hiervan mogen niet in strijd zijn met deze Statuten.

VII. Slotbepalingen

Artikel 33. Ongewijzigd art. 72. De gewijzigde Statuten treden in werking zodra daarop de Koninklijke goedkeuring zal zijn verkregen.

Artikel 34. Ongewijzigd art. 73.

Artikel 35. Ongewijzigd art. 74.

Artikel 36. Ongewijzigd art. 75; achter „Statuten” invoegen: „en het Huishoudelijk Reglement”.

c. alle artikelen uit de Statuten, voor zover zij niet onder het in b genoemde zijn begrepen, vervallen.

d. opgemaakt is een Huishoudelijk Reglement, dat aldus luidt:

1. Leden, Contribuanten, Donateurs en Begunstigers

Artikel 1

De eerste Secretaris geeft aan hen, die als lid, corresponderend lid, student-lid, donateur, begunstiger of contribuant zijn toegelaten, daarvan schriftelijk kennis. Aan hen, die tot erelid zijn benoemd wordt een door de Voorzitter en de eerste Secretaris ondertekend diploma uitgereikt.

Het lidmaatschap wordt geacht te zijn ingegaan op 1 Januari van het verenigingsjaar.

Artikel 2

Opzegging van het lidmaatschap moet schriftelijk geschieden aan het adres van de eerste Secretaris, die hiervan bericht van ontvangst zendt. De opzegging wordt van kracht met ingang van het eerstvolgende verenigingsjaar, indien zij vóór 1 December van het lopende jaar is geschied; anders met ingang van het daarop volgende verenigingsjaar.

Het in het voorgaande lid van dit artikel gestelde geldt ook voor contribuanten, donateurs en begunstigers.

Artikel 3

De leden betalen een jaarlijkse contributie van fl 17,50. Dit bedrag wordt op aanvraag verlaagd tot fl 15,- voor de leden, die tevens lid zijn van de Nederlandse Dierkundige Vereniging.

De student-leden betalen een jaarlijkse contributie van fl 12,50, indien zij de Acta Botanica Neerlandica ontvangen, of fl 7,50, wanneer zij de Acta Botanica Neerlandica niet ontvangen.

De contributie der contribuanten bedraagt fl 3,- jaarlijks.

Het Bestuur is gemachtigd in bijzondere omstandigheden voor bepaalde leden een lagere contributie vast te stellen.

De bijdrage van donateurs bedraagt fl 100,- tot fl 250,- jaarlijks; begunstigers dragen jaarlijks een door henzelf te bepalen bedrag, boven fl 250,- bij.

Artikel 4

Ereleden en corresponderende leden ontvangen de uitgaven der Vereniging kosteloos.

Artikel 5

Indien een lid na herhaalde aanmaning binnen een door het Bestuur te bepalen tijd niet aan zijn financiële verplichtingen voldoet, kan de ledenvergadering op voorstel van het Bestuur hem van het lidmaatschap vervallen verklaren.

II. Het Bestuur

Artikel 6 (oud art. 17).

Artikel 7 (oud art. 18).

Artikel 8 (oud art. 20).

Artikel 9 (oud art. 19 en 14); hierin wijzigen:

„59^a in „25^a“; weglaten:

„De vergadering stelt dat verslag vast. Hij zendt de naamlijst der leden en het vastgestelde verslag aan de na te noemen Redactie-Commissie voor het Nederlandsch Kruidkundig Archief“.

Invoegeen: Hij stelt het jaarboek van de Vereniging samen, waarin zijn opgenomen het jaarverslag, de verslagen van de Permanente Commissies, de verslagen van de ledenvergaderingen en andere vergaderingen, de rekening en verantwoording van de Penningmeester, de begroting en de naamlijst der leden. Hij draagt zorg voor het uitgeven van het jaarboek.

Artikel 10 (oud art. 15).

Artikel 11

De Penningmeester sluit ultimo December van elk jaar de boeken af. In een Bestuursvergadering, die aan de in artikel 25 der Statuten bedoelde ledenvergadering voorafgaat, legt hij rekening en verantwoording af van zijn beheer in het afgelopen jaar en geeft hij een overzicht van de samenstelling van het kapitaal der Vereniging. Het Bestuur legt deze gegevens in verkorte vorm voor aan de hierboven bedoelde ledenvergadering.

De Penningmeester int zo spoedig mogelijk de contributies der leden. Wordt de contributie ook na herhaalde aanmaning niet voldaan, dan geeft hij daarvan kennis aan het Bestuur, dat de nalatige met inachtneming van art. 5 van dit Huishoudelijk Reglement van het lidmaatschap vervallen kan verklaren.

De Penningmeester legt in de jaarvergadering de rekening en verantwoording van zijn beheer over het vorige jaar over. Hij ontwerpt een voorlopige begroting der uitgaven. Hierin worden in elk geval afzonderlijk vermeld:

- a. de algemene huishoudelijke uitgaven;
- b. die voor het tijdschrift en de verdere uitgaven der Vereniging, afzonderlijk begroot;
- c. die voor de Bibliotheek;
- d. die voor het Herbarium.

Artikel 12

Twee leden, door de jaarvergadering aan te wijzen, zien alle bescheiden, die op het beheer van het kapitaal der Vereniging betrekking hebben bij de Penningmeester na; eveneens de rekening en verantwoording met de daarbij behorende bewijsstukken. Zij rapporteren daaromtrent aan de volgende ledenvergadering.

Wordt de rekening goedgekeurd, dan dechargeert de vergadering de Penningmeester van zijn beheer.

III. De Permanente Commissies

Artikel 13 (oud art. 21); hierin te wijzigen:

Er zijn acht commissies:

a. de Commissie van Redactie voor de Acta Botanica Neerlandica;

b. de Commissie voor de Bibliotheek en het Herbarium en het Archief.

enz., waarbij c in b veranderd wordt, enz.

Artikel 14 (oud art. 22); hierin wijzigen:

„59” in „25 der Statuten”.

Artikel 15 (oud art. 23); hierin wijzigen:

c in b, enz.

„in de commissie genoemd sub a één om het andere jaar” in „in de commissie genoemd sub a elk jaar één van de leden, die geen bestuurslid van de Vereniging zijn”.

Artikel 16 (oud art. 24); hierin wijzigen:

„.....voor het Recueil des Travaux Botaniques Neerlandais.....” in „.....voor de Acta Botanica Neerlandica.....”.

„51” in „dit Huishoudelijk Reglement”.

Artikel 17 (oud art. 25); hierin wijzigen:

„.....vanwege het Bestuur aan de Commissie van Redactie voor het Nederlandsch Kruidkundig Archief toegezonden” in „.....in het jaarboek opgenomen”.

Artikel 18

De Commissie van Redactie bestaat uit de Voorzitter, de eerste Secretaris en de Penningmeester der Vereniging; de Directeuren in art. 16 van dit Huishoudelijk Reglement bedoeld en minstens 5 leden, niet tot deze Directeuren behorend.

De Commissie is belast met de zorg voor de uitgave van de Acta Botanica Neerlandica en voor alle verdere uitgaven, die door de ledenvergadering aan haar worden opgedragen.

Artikel 19 (oud art. 28).

Artikel 20 (oud art. 29).

Artikel 21 (oud art. 30).

Artikel 22 (oud art. 31).

Artikel 23 (oud art. 32).

Artikel 24 (oud art. 33).

Artikel 25 (oud art. 34); hierin het derde lid als volgt wijzigen:

Het Bestuur is bevoegd contribuanten bij de Commissie voor de Phytopathologie toe te laten.

Artikel 26 (oud art. 35).

Artikel 27 (oud art. 36).

Artikel 28 (oud art. 37).

Artikel 29 (oud art. 38).

IV. Stemmingen

Artikel 30

Behoudens het in de Statuten in art. 30, 33 en 34 bepaalde geldt ten aanzien van stemmingen het volgende:

hierna laten volgen oud art. 67, waarin gewijzigd:

„12^e in „15 der Statuten”,

„22^e in „14 van het Huishoudelijk Reglement”.

V. Ledenvergaderingen

Artikel 31

Jaarlijks worden minstens 2 ledenvergaderingen gehouden, waarvan één de jaarvergadering is. De overige vergaderingen zijn in het bijzonder bestemd voor het houden van wetenschappelijke voordrachten en de bespreking daarvan.

Artikel 32 (oud art. 61).

Artikel 33 (oud art. 63); hierin wijzigen:

„ledenvergadering” in „vergadering”.

Artikel 34 (oud art. 64).

Artikel 35 (oud art. 65); hierin wijzigen:

„....en zendt dit aan de Commissie van Redactie voor het Nederlandsch Kruidkundig Archief” in „....zendt dit aan de eerste Secretaris ter opneming in het jaarboek van de Vereniging”.

Artikel 36 (oud art. 66).

VI. Tijdschriften en verdere uitgaven

Artikel 37 (oud art. 51); hierin wijzigen:

„.....twee tijdschriften” in „.....een tijdschrift, de Acta Botanica Neerlandica”.

Artikel 38

Dit tijdschrift verschijnt in 3-maandelijks afleveringen, die jaarlijks een deel vormen. Het bevat wetenschappelijke bijdragen, die in het Nederlands geschreven kunnen zijn, doch bij voorkeur in het Engels, Frans of Duits.

De schrijvers staan het auteursrecht der opgenomen stukken af aan de Vereniging.

Artikel 39 (oud art. 55); hierin wordt het eerste lid aldus gewijzigd:
„Het tijdschrift is voor de in art. 6 der Statuten genoemde leden der Vereniging verkrijgbaar tegen een prijs, die belangrijk lager is dan de handelsprijs, Corresponderende leden en ereleden ontvangen de Acta kosteloos.”
Het laatste lid blijft ongewijzigd, echter wordt „56” vervangen door „40 van dit Huishoudelijk Reglement”.

Artikel 40 (oud art. 56); hierin wijzigen:
„.....der tijdschriften” in „.....van het tijdschrift”.

Artikel 41

Jaarlijks voor 1 Juni wordt een jaarboek van de Vereniging uitgegeven. De eerste Secretaris is met deze uitgave belast. Het bevat in ieder geval: (hierna volgen de punten a tot en met i van art. 52 oud).

De ereleden, corresponderende leden, leden, donateurs en begunstigers ontvangen het jaarboek kosteloos.

VII. Verzamelingen

Artikel 42 (oud art. 57).

VIII. Excursies

Artikel 43 (oud art. 68).

Artikel 44 (oud art. 69).

Artikel 45 (oud art. 70).

Artikel 46 (oud art. 71).

Reglement voor de Commissie voor Bibliotheek, het Herbarium en het Archief

Hierin wordt alleen gewijzigd:

„art. 1-6” in „Art. 47-52”.

„art. 4” in „art. 50 van dit Huishoudelijk Reglement”.

Reglement voor de Commissie voor het Floristisch onderzoek van Nederland

Hierin wordt gewijzigd:

„oud art. 1-8” in „art. 53-60”.

„art. 22” in „art. 14 van dit Huishoudelijk Reglement”.

Reglement voor de Commissie voor Biosociologie en het Veenonderzoek van Nederland

Hierin wordt gewijzigd:

„art. 1-3” in „art. 61-63”.

„art. 22 der Statuten” in „art. 14 van dit Huishoudelijk Reglement”.

Reglement der Commissie voor de bescherming van de Wilde Flora

Hierin wijzigen:

„art. 1-2” in „art. 64-65”.

„art. 38 der Statuten” in „art. 29 van dit Huishoudelijk Reglement”.

Reglement der Commissie voor de Phytopathologie

Hierin wijzigen:

„art. 1-3” in „art. 66-68”.

„art. 22 der Statuten” in „art. 14 van dit Huishoudelijk Reglement”.

Reglement der Commissie voor de Plantenphysiologie.

Hierin wijzigen:

„art. 1-3” in „art. 69-71”.

„art. 22 der Statuten” in „art. 14 van dit Huishoudelijk Reglement”.

Reglement der Commissie voor de Plantensystematiek en •geografie

Hierin wijzigen:

„art. 1-3” in „art. 72-74”.

„art. 22 der Statuten” in „art. 14 van dit Huishoudelijk Reglement”.

Reglement voor de Commissie van Redactie voor de Acta Botanica Neerlandica

Het Bestuur zal in overleg met deze commissie een voorstel voor een regeling indienen.

e. Het Bestuur verkrijgt machtiging van de ledenvergadering al die wijzigingen in de Statuten of het Huishoudelijk Reglement aan te brengen, die eventueel door het Departement verlangd zullen worden ter verkrijging van de goedkeuring.

Bij het bespreken van deze statutenwijzigingen worden de volgende punten besproken:

Op de vraag van Dr BOTTELIER, of alle leden de Acta zullen ontvangen, antwoordt de Voorzitter bevestigend. Alleen echtgenoten van leden, die ook lid van de Vereniging zijn, ontvangen het tijdschrift niet; hun contributie is dan bepaald op f 12,50.

Verder vraagt Dr BOTTELIER of het niet gewenst zou zijn een afzonderlijke boekhouding voor de Acta Botanica Neerlandica te voeren. De Vereniging ontvangt immers subsidies van laboratoria voor het uitgeven van dissertaties. Nu worden deze subsidies in de algemene begroting opgenomen. Het komt hem noodzakelijk voor deze subsidies op een afzonderlijke rekening te boeken en hierin óók op te nemen een post „subsidie van de Bibliotheek voor het uitgeven van de Acta”. Immers een aantal exemplaren van de Acta worden als ruilobject verzonden, en dit komt de Bibliotheek ten goede, maar drukt op de exploitatie-rekening van het tijdschrift.

De Penningmeester antwoordt hierop, dat het splitsen van de algemene rekening in afzonderlijke exploitatie-rekeningen bezwaren heeft. Dit zou de boekhouding veel ingewikkelder maken en volgens hem geen bijzondere voordelen bieden. Dr BOTTELIER merkt op, dat het Botanisch Laboratorium te Utrecht gedurende een aantal jaren heeft bijgedragen voor een totaal-bedrag van omstreeks fl 2.500,-. Deze post komt niet in de exploitatie-rekening van het tijdschrift voor. Moeilijkheden kunnen nu ontstaan, wanneer dit laboratorium enkele dissertaties in één jaar zou willen uitgeven.

De eerste secretaris wijst er op, dat deze moeilijkheden ook bij de bestaande toestand ondervangen kunnen worden. Het inrichten van een afzonderlijke exploitatie-rekening voor het tijdschrift heeft volgens hem het bezwaar, dat men zeer moeilijk de waarde van de ruilabonnementen kan schatten: is dit een evenredig deel van de totale kosten of slechts het deel dat betaald moet worden als extra nummers? Dit laatste zou slechts een zeer laag bedrag zijn. Dr VAN SOEST wijst er nog op, dat de Bibliotheek geen typisch floristische bibliotheek is, maar boeken en tijdschriften bevat, die voor alle richtingen van belang zijn.

Na deze discussie wordt besloten de bestaande toestand te handhaven.

Tenslotte besluit de vergadering op voorstel van het Bestuur Mevr. Dr J.H.H. VAN DE LAAR-v.d.MEER als lid te schrappen, daar grote achterstand is ontstaan in het betalen der contributie. Herhaalde verzoeken om dit aan te zuiveren hebben geen resultaat gehad.

Hiermee sluit de Voorzitter de ochtend-bijeenkomst.

Te twee uur wordt de vergadering door de Voorzitter heropend. In deze bijeenkomst houdt Dr C.G.G.J. VAN STEENIS een voordracht over *Rheophyten* en houdt Mej. Dr Joh.C. WENT een causerie over *Boomziekten in de Verenigde Staten*. Een levendige discussie volgde op elk van deze voordrachten.

Niets meer aan de orde zijnde sluit de Voorzitter, na de sprekers dankte hebben gebracht voor hun interessante en belangrijke mededelingen, de bijeenkomst.

17 Mei 1952.

De Voorzitter,
E. VAN SLOGTEREN

De 1ste Secretaris,
A.W.H. VAN HERK

**ALGEMENE LEDENVERGADERING gehouden op
Zaterdagavond 17 Mei 1952 te Amsterdam**

Aanwezig waren 17 leden, namelijk: A.T.J. BIANCHI, BREMEKAMP, BREMER-REINDERS, BREMER, DOCTERS VAN LEEUWEN, HEIMANS, VAN HERK, JAARVELD, E. VAN SLOGTEREN, VAN SLOOTEN, STOMPS, VERMEULEN, DE VISSER SMITS, VAN WAESBERGHE, S.J., H.C.D. DE WIT, ZEYLEMAKER en 1 onleesbaar.

De Voorzitter, Dr E. VAN SLOGTEREN, opent om half acht de vergadering.

De Notulen worden voorgelezen en goedgekeurd. Naar aanleiding hiervan zegt de Voorzitter, dat het Bestuur ook nog gaarne een bespreking wenst met de Redactie van FLORA NEERLANDICA.

Verder deelt hij mede, dat de Commissie voor het nazien van Rekening en Verantwoordings en van de Bescheiden van de Penningmeester op 13 Mei 1952 deze heeft gecontroleerd en juist bevonden, en voorstelt de Penningmeester te dechargeren. Dit geschiedt ter vergadering, met dank aan de penningmeester voor zijn zorgvuldig beleid.

Dan herdenkt de Voorzitter ons overleden medelid Dr J. BRAND. Onder de Floristen was hij een zeer actief lid. Zelfs enige dagen voor zijn overlijden heeft hij nog een brief geschreven aan de Commissie voor de Floristiek. Op een vraag van HEIMANS, of er nog acht geslagen is op het laatste verzoek van BRAND (over het onteigenen van bepaalde terreinen door de militairen) deelt VAN HERK mede, dat hij er naar geïnformeerd heeft. Het Departement van Defensie heeft medegedeeld, dat men deze terreinen niet wil onteigenen; men wil er alleen overheenlopen.

Het aspirant-lid, Ir W.G. BEEFTINK, voorgesteld per convocatie, wordt bij acclamatie benoemd, evenals de heer ALBERTS, die met dispensatie van Art. 2, 1e lid, op de vergadering wordt voorgesteld door de leden VAN HERK, JANSEN, KLOOS.

Daar wordt overgegaan tot het vaststellen van een Reglement voor de Commissie van Redactie van de Acta Botanica Neerlandica. Van het voorstel van het Bestuur worden de eerste vier artikelen zonder discussie aangenomen. Over de artikelen 5 en 6 worden enige opmerkingen gemaakt (VAN STEENIS, VAN DIJK, HEIMANS). Na ampele bespreking wordt aan het voorgestelde artikel 5 nog iets toegevoegd, artikel 6 wordt enigszins anders opgesteld.

Over artikel 7 is een schrijven binnengekomen van KONINGSBERGER. Het blijkt evenwel, dat hij de bedoeling van het Bestuur heeft misverstaan. De Voorzitter stelt voor, het artikel te handhaven zoals het gesteld is, met nog een kleine, door BREMEKAMP voorgestelde toevoeging. De vergadering is het hiermee eens en accepteert ook gaarne deze toevoeging. Tevens wordt besloten, dat de 1e Secretaris de juiste redactie van de artikelen zal opstellen.

Het Reglement luidt tenslotte aldus *):

*) Enkele kleine veranderingen van redactie voorbehouden.

Artikel 1

De Voorzitter van de Vereniging is tevens Voorzitter van de Commissie van Redactie.

Artikel 2

De Commissie wijst in haar eerste vergadering van ieder jaar uit haar midden twee secretarissen aan en regelt hun werkzaamheden.

Het Secretariaat voert de correspondentie met de uitgever en met de auteurs van de ter publicatie aangeboden artikelen. Het is met de zorg van de correctie belast. Aan het secretariaat is tevens opgedragen de commissievergaderingen bijeen te roepen en de notulen van deze bijeenkomsten te houden.

Artikel 3

De Commissie is belast met de algemene verzorging van het tijdschrift. Zij alleen beslist over het opnemen van aangeboden artikelen, behoudens het in art. 7 bepaalde. Zij regelt ook, hoe de artikelen moeten worden aangeboden.

Artikel 4

De Commissie vergadert ieder kwartaal minstens één maal, voorts wanneer het Bestuur hiertoe besluit volgens het in art. 27 van het Huishoudelijk Reglement bepaalde en verder zo dikwijls de Voorzitter of drie van haar leden het verlangen daartoe aan het secretariaat hebben kenbaar gemaakt. Dit roept daarna in overleg met de Voorzitter binnen een maand een vergadering bijeen.

Artikel 5

De Commissie is verantwoordelijk voor de uitgaven, verricht in verband met het uitgeven van het tijdschrift. Zij mag het totaal bedrag, bij de begroting voor het uitgeven van het tijdschrift beschikbaar gesteld, niet overschrijden zonder machtiging van het Bestuur.

Artikel 6

De voor de Acta Botanica Neerlandica bestemde bijdragen worden bij voorkeur in de Engelse, Franse of Duitse taal geplaatst. De Redactie kan echter toestaan, dat een artikel, in het Nederlands gesteld, wordt opgenomen.

Artikel 7

Bijdragen voor de Acta, die worden aangeboden op grond van een contract als bij art. 37 van het Huishoudelijk Reglement bedoeld, worden geplaatst voorzover ze in taalkundig aanvaardbare vorm zijn gesteld en voorzover het in het contract vastgestelde bedrag aan kosten niet wordt overschreden. Is dit laatste wel het geval, dan kunnen deze bijdragen alleen geplaatst worden na goedkeuring door de Commissie.

Om omstreeks kwart over acht begint het wetenschappelijk gedeelte van de vergadering.

De eerste voordracht werd gehouden door Dr G. BREMER over: *Vermeerdering van chromosomenaantal bij soortsbastaardering van Saccharum in verband met de embryozakontwikkeling*.

Een kort verslag van deze voordracht is opgenomen in de rubriek Mededelingen (blz. 36).

Deze voordracht werd verduidelijkt door projectie van tal van microscopische preparaten. Na afloop was er discussie, waaraan werd deelgenomen door de leden VERMEULEN, DE VISSER SMITS en VAN WAESBERGHE.

Na de pauze sprak Dr H.C.D. DE WIT over: *Rumphius herdacht (1702-1952)*. Een résumé van deze voordracht is opgenomen in de rubriek Mededelingen (blz. 37).

Na deze interessante voordracht over leven en werken van Rumphius sloot de Voorzitter om kwart over 10 de vergadering, na dank gebracht te hebben aan de beide sprekers.

15 Juni 1952.

De Voorzitter
E. VAN SLOGTEREN

De 2e Secretaresse
A. JAARVELD

ZOMERVERGADERING EN -EXCURSIE gehouden op Zaterdag 14 en Zondag 15 Juni 1952 te Lisse

De zomervergadering werd dit jaar gehouden te Lisse op Zondag 15 Juni. Aan de vergadering ging een excursie vooraf op Zaterdagmiddag naar het Nationale Park „De Kennemer Duinen”.

Aan deze excursie, welke werd gehouden onder leiding van de leden Dr S.E. DE JONGH en R.J. SANDERS, namen 30 personen deel. Zij was zeer geslaagd, hetgeen behalve aan de leiders mede te danken was aan de medewerking, welke Ir E.C.M. ROODERKERK bij de voorbereiding heeft verleend en waarvoor hem op deze plaats hartelijk dank wordt gebracht.

Na afloop van de excursie werd een gemeenschappelijke maaltijd gehouden in Hotel Noordzee te Noordwijk aan Zee, waar ook de avond genoegelijk werd doorgebracht, aangezien het weer niet lokte voor een strandwandeling.

Zondagmorgen 15 Juni vertrokken de leden, die in Noordwijk gelogeerd hadden, per auto naar Lisse naar het Laboratorium voor Bloembollenonderzoek. Ook de leden, die uitsluitend de vergadering en demonstraties wilden meemaken en niet aan de excursie hadden deelgenomen, arriveerden daar succesvol.

De belangstelling was zeer groot. Aanwezig waren 10 introducés, de dames en heren: BALLING, BEIJER-LIKES, CRANIN, CREMER, COCH, DAHMEN, PULLE-STARKE, RIJVEN-SIMONS, VAN SLOGTEREN-VAN LESSEN en VROOM, en 80 leden, namelijk: ANDREAS, ARISZ, VAN ARKEL, BANNING, BECKER, BELS-KONING, BEIJER, A.J. BIANCHI, BLOK, S. DE BOER, BOSCHAEERT, BOTTELIER, VANDEN BROEK, BOSUIZEN, BRUINSMA, DE BRUYN, DE BRUYN CUBOTER, LE COSQUINO DE BUSSY, DALSUM, DOCTERS VAN LEEUWEN, W. VAN DIJK, DIJKSTRA, A. GORTER, GOOSSENS,

HAANS, HARMSSEN, HEGNAUER, HEIMANS, VAN HELL, VAN HERK, HOUWINK, HUISINGA, JAARSVELD, DE JONGE-SIMON THOMAS, JONKER, KESSLER, KLEINHOONTE, KONINGSBERGER, KOSTER, KREULEN, VAN DER LAAN, LAM, LENS, J.C.LINDEMAN, LODDER, LÖHNIS, MAAS GEESTERANUS, A.M.W.MENNEGA, E.A.MENNEGA, MONOD DE FRODEVILLE, VAN NIE, MARIS MC ARTHUR-HESPE, OOMEN, OOSTERHUIS, PIECK, PULLE, QUAK, REINDERS-CORBACH, E.VAN SLOGTEREN, SLOOTWEG, SMULDERS, VAN SOEST, SPIERENBURG, STAFLEU, VAN DEN STEENHOVEN, VAN STEENIS, STEGWEE, STIENSTRA, TAMMES, DE VISSER SMITS, WASSINK-VAN LUMMEL, VAN WAESBERGHE S.J., WESTERDIJK, WILBRINK, VAN WIJK, DE ZEEUW, ZEYLEMAKER, en drie onleesbaar.

Bij aankomst werden allen onthaald op koffie in de met fraaie bloemstukken versierde hal van het nieuwste gebouw van het laboratoriumcomplex. Daarna begaf men zich naar de practisch en smaakvol ingerichte nieuwe vergaderzaal, waar de Voorzitter, Dr E.VAN SLOGTEREN, Directeur van het laboratorium, omstreeks elf uur de vergadering opende en, niet alleen als voorzitter, doch eveneens als gastheer, de aanwezigen welkom heette.

Allereerst moest hij enige droeve feiten memoreren en wel het overlijden van een drietal leden. In de eerste plaats Dr H.L. VAN DE SANDE BAKHUYZEN, die nog niet zo lang lid was van onze Vereniging; hij was een bekwaam physioloog, werkzaam aan de Landbouwhogeschool te Wageningen. Dan Dr F.W.T.HUNGER, ons oudste lid – hij trad in 1895 als lid tot onze Vereniging toe – die zich vooral bewoog op het gebied van de geschiedenis der botanie. En als derde Dr Ir A.W.KLOOS JR, lid sinds 1912, zeer bekwaam florist, die gedurende vele jaren zijn capaciteiten in dienst van onze Vereniging stelde en verschillende malen, in afwisseling met Dr P.JANSEN, als penningmeester optrad. Ook in het huidige Bestuur bekleedde hij de functie van penningmeester en was een prettig en zeer gewaardeerd mede-bestuurslid. Het verlies, dat de Vereniging door zijn heengaan lijdt, is nog niet te beseffen. Het Bestuur was aanwezig bij zijn crematie, waar de Voorzitter enige afscheidswoord sprak en zijn betekenis voor de Koninklijke Nederlandse Botanische Vereniging schetste.

De Vergadering nam daarna enkele minuten stilte in acht ter gedachtenis aan de overledenen.

Daarna wees de Voorzitter erop, dat door het overlijden van KLOOS moeilijkheden waren ontstaan: de rekeningen waren namelijk geblokkeerd. Er moest dus tussentijds een nieuwe penningmeester benoemd worden. KLOOS zelf had steeds gehoopt, dat Ir J.L. VAN SOEST het penningmeesterschap zou willen waarnemen. Deze was daartoe wel bereid. De Voorzitter stelde daarom voor, Ir J.L. VAN SOEST tot penningmeester te benoemen. De Vergadering nam dit voorstel bij acclamatie aan. De heer VAN SOEST verklaarde de benoeming aan te nemen.

Aangaande het tijdschrift kon de Voorzitter mededelen, dat het tweede nummer van de *Acta Botanica Neerlandica* zou verschijnen op 1 juli 1952.

Daarna ging men over tot het *Wetenschappelijk gedeelte* van de vergadering. De Directeur en een drietal medewerkers van het Instituut gaven in de volgende voordrachten een overzicht van de verschillende werkzaamheden, die in het laboratorium worden verricht, nl.:

Dr E. VAN SLOGTEREN: Inleiding over het werk in het Laboratorium voor Bloembollenonderzoek.

Dr J.J. BEIJER: Het onderzoek betreffende periodiciteit van bloembollen.

Drs A.F.G. SLOOTWEG: Het wortelrot in Narcissen.

Ir D.H.M. VAN SLOGTEREN: Virusonderzoek en Serologische Diagnostiek.

Tot slot vertoonde de Directeur een kleurenfilm, die een duidelijk en uitvoerig beeld gaf van de werkzaamheden, vooral op virologisch gebied.

Na deze belangwekkende uiteenzettingen werd de deelnemers een koffie-maaltijd aangeboden, die een feestelijke sfeer hielp teweegbrengen en die onder het geanimeerd discours van de talrijke aanwezigen werd genoten.

Des middags maakte men in groepen een rondgang door de diverse laboratoriumgebouwen, waar allerlei demonstraties werden gehouden, in aansluiting op de 's morgens gehouden voordrachten. Verder kon men ook de gehele inrichting bezichtigen en de vele apparaten, waarmee het laboratorium zo modern is geoutilleerd. Men zag er bij voorbeeld het uitgebreide instrumentarium van de serologische afdeling van het viruslaboratorium; het electronenmicroscop; de klimaatcellen, waarbij temperatuur en vochtigheid automatisch geregeld worden; de verschillende kassen, enz.

Aan het einde van deze zeer interessante middag kwamen de deelnemers weer tesamen om nog een kopje thee te drinken. Tijdens deze thee sprak Dr V.J. KONINGSBERGER namens alle aanwezigen de hartelijke dank uit jegens onze gastheer en zijn medewerkers, voor deze uiterst geslaagde zomervergadering, voor het vele en belangwekkende, wat wij te horen en te zien gekregen hadden, voor de prima organisatie van het geheel, waardoor ook werkelijk ieder van de vele belangstellenden alles had kunnen zien, en bovendien voor het gulle en gastvrije onthaal.

Hierna sloot de Voorzitter-gastheer omstreeks vijf uur de vergadering.

1 Februari 1953.

De Voorzitter

E. VAN SLOGTEREN

De 2e Secretaresse

A. JAARVELD

MEDEDELINGEN

Dr G. BREMER:

Vermeerdering van chromosomenaantal bij soortsbastaardering van Saccharum in verband met embryozakontwikkeling.

(Kort verslag van de lezing door Dr G. BREMER gehouden op 17 Mei 1952 te Amsterdam)

In 1920 vond spreker, dat bij kruising van suikerriet, *Saccharum officinarum* ($2n = 80$), als vrouwelijke plant in de kruising, met glagah, *S. spontaneum* ($2n = 112$) als mannelijke plant, soortsbastaarden ontstaan, die niet, zoals te verwachten was, een somatisch chromosomenaantal van $40 + 56 = 96$ bezitten, doch een aantal van $136 = 40 + 40 + 56$. Overeenkomstige vermeerderingen in chromosomenaantal werden later bij tal van andere soortsbastaarden van *Saccharum* gevonden.

De eenvoudigste verklaring zou zijn, dat *S. officinarum* eicellen vormt zonder reductiedeling. In de megaspore-moedercellen werd echter geregeld reductiedeling gevonden. Ook de talrijke kleurschakeringen der stengels, voorkomend bij verschillende individuen uit het zaaisel van een suikerriet pluim, die gekruist is met glagah, wijzen op segregatie van factoren bij het suikerriet.

Het bleek, dat de chromosomenvermeerdering, die bij deze soortsbastaarden gevonden wordt, berust op een verdubbeling van het aantal chromosomen bij de suikerriet-moeder, die in de kruising gebruikt is.

Bij suikerriet worden namelijk naast haploide embryozakken, embryozakken gevormd welke diploid zijn. De haploide embryozakken ontstaan op normale wijze. De diploide ontstaan uit een megasporogenese, die een afwijkend verloop heeft. Nadat in de megaspore-moedercellen normale reductiedeling plaats gehad heeft, waardoor dus haploide megasporen ontstaan, heeft in de binnenste, chalazale, megaspore een verdubbeling van de chromosomen binnen de kern plaats, door overlangse splitsing van alle chromosomen, waardoor de kern dus diploid wordt.

Dit is dus een merkwaardig geval van endomitose. In meer zeldzame gevallen heeft echter reeds endomitotisch splitsing van de chromosomen in de binnenste megaspore-diadecel plaats. Deze splitsing kan zich dan nog éénmaal herhalen. Zonder dat daarna nog een tweede meiotische deling plaats heeft, groeit deze chalazale diadecel dan tot een tetraploide embryozak uit.

Het aldus ontstaan van haploide, diploide en tetraploide embryozakken kon getoetst worden aan kerndelingen binnen de zich verder ontwikkelende embryozakken, waarbij wederom haploide, diploide of tetraploide kernen gevonden werden.

In verband hiermede werden enige gevallen van soortsbastaarden bij andere plantengeslachten genoemd, waarbij chromosomenvermeerdering wellicht op overeenkomstige wijze zal plaats hebben.

Dr H.C.D. DE WIT:
RUMPHIUS herdacht (1702 – 1952)

(Kort verslag van de lezing door Dr H.C.D. DE WIT gehouden op 17 Mei 1952 te Amsterdam)

GEORG EVERHARD RUMPF – RUMPHIUS voor de geleerde wereld – stierf op 15 Juni 1702, dat is dus 250 jaar geleden en dit moge aanleiding zijn hem nog eens in het bijzonder te gedenken. Hij woonde en werkte vooral als Koopman in dienst van de O.L. Compagnie op Ambon, waar hij in 1653 aankwam en bleef tot zijn dood. Zijn werk als Compagnies dienaar was voor hem, hoe bekwaam hij zich ook toonde, bijzaak. Hij wijdde zich met hart en ziel aan het natuurhistorisch onderzoek van de Molukken en liet over de flora van die Archipel een werk na, dat nimmer overtroffen of zelfs maar geëvenaard werd: „Herbarium Amboinense” of „Ambons Kruidboek” (6 dln, 1741 – 1750, en 1 dl „Auctuarium”, 1755).

RUMPHIUS werd in 1627 geboren in het graafschap Solms. Naar geest en neiging ontplooidde hij zich als Hollander en men mag zich afvragen of hij misschien van moederszijde van Hollandse afkomst was. Na een onvrijwillig verblijf in Portugal – hij viel als soldaat der West Indische Compagnie, in wier dienst hij op valse voorspiegelingen was geraakt, in handen van de Portugezen – keerde hij naar Hanau terug, waar zijn ouders woonden. Daarop neemt hij dienst bij de O.L. Compagnie omdat hij naar Water Indiën wenste te gaan om de natuur daar ter plaatse te onderzoeken.

Op Ambon maakte hij voortdurend promotie maar de bitterste tegenslagen bleven hem niet bespaard. Dat hij werd gepasseerd als „Secunde”, de hoogste autoriteit onder de Gouverneur, is misschien minder belangrijk, behalve dan dat hij als troost voor de terugzetting een botanische tuin in eigendom verkreeg. Ook geeft de Compagnie hem de vrijheid, hoewel hij in haar dienst bleef, zijn wetenschappelijk onderzoek te behartigen.

In 1670 treft hem de zwaarste ramp die hem bij zijn werk treffen kon: hij verliest het licht in beide ogen. Vier jaar later komen bij een aardbeving zijn vrouw en jongste dochter om het leven. In 1687 verliest hij, bij de grote brand van Ambon al de oorspronkelijke tekeningen, die hij als illustratie van zijn Kruidboek, toen hij nog zien kon, eigenhandig had vervaardigd.

Met hulp van assistenten en vele vrienden slaagt hij er in de geleden schade goeddeels te herstellen. Onder hen verdienen PAUL AUGUST, zijn zoon, en G.G. JOAN MAETSUYCKER vooral genoemd te worden. Door de goede zorgen van G.G. J. CAMPHUYS bleven te Batavia daar vervaardigde copieën van het Herbarium Amboinense bewaard toen het origineel naar Nederland werd verscheept, een wijze maatregel want dit ging, met het schip waarin het werd vervoerd, te gronde. Een nieuwe poging slaagde en daarna rustte het manuscript ruim dertig jaren in de kantoren der O.L. Compagnie te Amsterdam waarna het in handen kwam van Professor J. BURMAN, Hoogleraar in de Botanie aan de Universiteit. Deze heeft het, uitstekend verzorgd, het licht doen zien.

Het Herbarium Amboinense is, uit hoofde van zijn uitzonderlijke kwaliteiten, een nog altijd veel geraadpleegd werk. Dat een boek van zo hoge ouderdom nog heden als een betrouwbare en uitvoerige bron voor modern wetenschappelijk onderzoek wordt gebruikt, is een unicum.

Er bestaan een aantal belangrijke studies, die zich uitsluitend of vooral met het Herbarium Amboinense bezig houden. Her werk is in meer dan duizend wetenschappelijke botanische publicaties geciteerd. Het onderzoek ingesteld naar de door RUMPHIUS beschreven planten kostte het leven van de Nederlandse geleerde, Dr J.G. BOERLAGE, die als botanicus van 's-Lands Plantentuin te Buitenzorg in 1900 naar de Molukken reisde en daar stierf; zo ook, in 1913, de Amerikaanse plantkundige, Dr C.B. ROBINSON, die werkte voor het Herbarium te Manila.

Als geleerde had RUMPHIUS een goed inzicht in natuurlijke verwantschappen, al ging hij uit van een geheel andere gedachtenwereld als de hedendaagse systematicus. Hij was een begaafd phytograaf, die de gave had op treffende wijze niet alleen, maar ook met verbazende accuratesse zijn voorwerpen te beschrijven. Hij had grote belangstelling voor de ecologie van de planten, ook voor hun biologie en voor hun economische betekenis.

RUMPHIUS was behalve botanicus een zeer veelzijdig geïnteresseerd onderzoeker, die zowel op taalkundig als historisch gebied erkende bekwaamheden bezat; hij was tevens vestingbouwkundige.

Hij schreef o.a. nog een drie-delig Dierboek, dat is verloren gegaan, en een „Rariteitkamer”. Dit laatste werk is wel gepubliceerd (1705). Het bevat vooral studies over weekdieren, koralen, stekelhuidigen, etc.

Enkele karaktertrekken van de mens RUMPHIUS kunnen we leren begrijpen door een degelijk onderzoek van zijn werken. Hij toont zich een rustig en evenwichtig man, wars van uiterlijk vertoon, neigend naar het sobere en van een nooit aflatende volharding en werklust. Hij heeft een levendig gevoel voor humor, is tolerant, een goed mensenkenner, steeds kritisch in opbouwende zin, d.w.z. hij verwerpt slechts na koele vergelijking van pro en contra. Hij experimenteert en doet, waar mogelijk, empirisch onderzoek.

De figuur van de pionier RUMPHIUS rijst voor ons op als die van een groot baanbreker in de ontwikkeling der Malesische botanie, groot als geleerde en groot als mens.

**LIJST van de LEDEN der
Koninklijke Nederlandse Botanische Vereniging**

September 1953

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LEDEN ¹⁾

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 Dr E.F. Drion (1930), 's-Gravenhage, Groenhovenstraat 14.
 Mej. C.E. Droogleever Fortuyn (1951), Amsterdam-Z., Stadionkade 139.
 P. Dullemeier (1948), Leiden, Fred. van Eedenlaan 27.
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 P.J.S. van Dijk (1937), Boskoop, Reyerskoop 221.
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 W.A. van Eck (1950), Michigan (U.S.A.), Soil Science Department, Michigan State College; (vac. adres: Zwolle, Brederostraat 30).
- H.J. van Eekeren (1917), Zeist, Eikenlaan 2.
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 Ir G. van den Ende (1951), Baarn, Sophialaan 37.
 J.H. Engelen (1947), Groningen, Moesstraat 6B.
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 G.L. van Eyndhoven (1943), Haarlem, Floraplein 9.
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- D. Franke (1950), Eernewoude.
 R. Frantzen (1922), Rotterdam-Z., Leede 22.
 Mej. R. de Fremery (1952), Utrecht, Prins Hendriklaan 66.
 A. Fuchs (1953), Leiden, Rijn en Schiekade 115 c.
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 Mej. Dr A.M. Hartsema (1946), Wageningen, Dr Boeslaan 10.
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 Mej. G.R. van Hees (1948), Amsterdam-W., Paramaribostraat 22 II.
 Dr R. Hegnauer (1949), Leiden, Burggravenlaan 251.
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 Dr Ir F. Hekker (1944), Delft, Spoorsingel 99.
 Dr R.J. Helder (1942), Groningen, Jacob Catsstraat 108.
 Dr W.E. van Hell (1928), Voorburg, Rusthoflaan 8.
 J. Hendriks (1950), Amsterdam-O., Radioweg 42.
 Dr J.Th. Henrard (1912), Oegstgeest, Warmonderweg 54.
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 Dr W.C. van Heurn (1935), Wilp (bij Deventer), F 222, Huize „Kleine Noordijk“.
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 W.M. Heyl (1920), Oegstgeest, Fred. Hendriklaan 33.
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 Mej. Dr K.J. Hocke Hoogenboom (1917), Zonnemaire (Schouwen).
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 Mej. Dr T. Hof (1931), 's-Gravenhage, Lohengrinstraat 18.

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 H. Hoogendoorn (1942), Oudewater, Markt B 216.
 Dr R.D. Hoogland (1943), Nieuw Guinea.
 Mej. J.J.M. Hoymans (1953), Voorschoten, Elslaan 2.
 Dr J.G. ten Houten (1931), Wageningen, Englaan 6.
 Dr A.L. Houwink (1928), Delft, Prof. Schoemakerstraat 63.
 H.J. Hueck (1945), Delfgauw (post Delft), Vreedestraat 14.
 B. Huisinga (1948), Utrecht, Kromme Nieuwe Gracht 28.
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 Lange Raamsteeg 2.
 H.S.C. Huijsman (1927), Doetinchem, Ds van Dijkweg 16.
 J.A. Hijner (1947), Bergen op Zoom, p/a Mevr. Nuyten de Vreug, Boschstraat.
 Mej. E.J. Idenburg (1925), Utrecht, Jan van Scorelstraat 30 bis.
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 Mej. W. van Iterson (1938), Amsterdam-Z., Watteastraat 17 IV.
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 J.A. Janse (1943), Bennebroek, van Ittersumlaan 32.
 Dr P. Jansen (1902), Amsterdam-Z., Frans van Mierisstraat 128.
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 G.J. de Joncheere (1947), c/o van Ommeren N.V., Rotterdam, Westerlaan 10.
 Mevr. Wed. G.A. de Jonge-Simon Thomas (1950), Amsterdam-Z., Watteastraat
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 Mej. Dr L.C.P. Kerling (1927), Baarn, Amaliaan 13.
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 Mej. H.E. Kessler (1952), Utrecht, Wittevrouwensingel 71.
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 Mej. Dr A. Kleinhoonte (1916), Delft, Nieuwe Plantage 13.
 Mej. Dr C.H. Klinkenberg (1930), Wageningen, Hamelakkerlaan 3.
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 H. Konings (1953), Veenendaal, Lindenlaan 42.
 Dr V.J. Koningsberger (1917), Utrecht, Nieuwe Gracht 187.
 Ir P. Kooiman (1953), Delft, Oostsingel 203.
 G. Kooistra (1951), Amsterdam-N., Nibbixwoudstraat 13.
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- Mevr. J.M. Koops-Bos (1953), 's-Gravenhage, Zuiderparklaan 360.
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 Herbarium Bogoriense.
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 Mej. A.C. Kreulen (1915), 's-Gravenhage, Schoutenstraat 28.
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 E. Laarman (1947), Groningen, N.Kijk in 't Jatstraat 82.
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 Mej. Dr J. Lodder (1932), Delft, Nieuwe Plantage 34.
 J.C. Lodder (1948), Goes, Heernisseweg 193.
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 Deptm. van Plantkunde.
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 Mej. Dr A.M.W. Mennega (1946), Utrecht, Nachtegaalstraat 28 bis.

- E.A. Mennega (1948), Utrecht, Mariahoek 18.
 Mej. Dr M.G. Mes (1934), Pretoria (Transvaal), Universiteit van Pretoria, Afdeling Botanica.
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 Dr W. Meyer (1946), Bogor (Indonesia), Herbarium Bogoriense.
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 A.W. Moll (1934), Amsterdam-W., Bonairestraat 15.
 Ch. Monod de Froideville (1952), Bergen (N-H), Bergerweg 12.
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 Dr M.F. Mörzer Bruyns (1938), Utrecht, Maliebaan 109.
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 Dr Ir E.G. Mulder (1948), Groningen, Kamplaan 2.
 F. Muller (1940), Haarlem, Kleverparkstraat 75.
 Dr F.M. Muller (1927), Haren (Gr), Dilgtweg 28.
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 J. Muller (1950), Amsterdam-Z., Ruysdaelkade 15 II.
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 T.J. van Heyningen Nanninga (1927), Groningen, Paterswoldseweg 79a.
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 Mej. Dr M.F.E. Nicolai (1929), Leiden, Burggravenlaan 245.
 Mej. R. van Nie (1946), 's-Graveland, Ned. Herv. Pastorie.
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 H.Th. Nieuwenhuysen (1920), Utrecht, Geertebolwerk 33.
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 L.E. Nijenhuis (1949), Woerden, Nieuwstraat 17.
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 G.H. Oltheten (1935), Voorschoten, Leidseweg 317.
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Mevr. R.C. Reinders-Corbach (1947), Wassenaar, Middelweg 6.
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 R.J. Sanders (1946), Haarlem, Lorentzplein 28.
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 A. Schoenmakers (1930), Bilthoven, Nicolailaan 7.
 G. Scholten (1949), Maarssen, Klokjeslaan 20.
 Mej. H.D. Schotsman (1942), Haren (Gr), Rijksweg 330.
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 Mej. J.C. Schreuder (1933), Wageningen, Beatrixlaan 8.
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 P. Sevenster (1947), Leiden, Steenschuur 8.
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 Dr G. Stahel (1914), Quinten am Walensee (Schweiz).
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Mr W.A. Swets (1951), Hilversum, Sparrenlaan 20.
Mej. Dr A. Kaars Sijpesteyn (1942), Utrecht, van Hogendorpstraat 16.
Dr P.M.L. Tammes (1926), Den Helder, Seringenlaan 14.
Mej. Dr W. Terpstra (1948), Utrecht, Willem Barendsstraat 22 bis.
A.L. Thierens Jr (1938), Eindhoven, Sint Bonifaciuslaan 19.
Dr J.B. Thomas (1931), Utrecht, Laan van Minsweerd 51.
Dr T.H. Thung (1946), Wageningen, Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 4a.
Ir P. Tideman (1946), Bogor (Indonesia), Dj. Kedung Halang 11A.
Dr F. Tjallingii (1931), Venlo, Dr Blumenkampstraat 15.
A.J. van Tooren (1947), Groningen, Dr D. Bosstraat 7A.
Dr H.J. Toxopeus (1922), Wageningen, Prof. Ritzema Bosweg 49.
Mevr. Dr J. Uri (1949), 's-Gravenhage, Willemstraat 54.
Dr K.F. Vaas (1934), Bogor (Indonesia), Kepata Balai Penjelidikan, Perikan Darae, Djalan Tjekeumeeh 99.
Dr W.W. Varossieau (1937), 's-Gravenhage, Ant. Duyckstraat 117.
Dr R. van der Veen (1927), Eindhoven, Hazeloop 7.
J.H. Venekamp (1950), Amsterdam-Z., Amstelveense weg 89 III.
Dr H.J. Venema (1945), Wageningen, Englaan 2.
Mej. C. Venverloo (1950), Leiden, de Sitterlaan 3A.
Dr F. Verdoorn (1927), Waltham 54 (Mass., U.S.A.), P.O. Box 151.
G. Verkuil (1949), Maarssen, Emmaweg 3.
J.D. Verleur (1952), Amsterdam-O., Newtonstraat 32.
G. Vermeulen (1927), Rotterdam-C., Statensingel 120A.
Dr P. Vermeulen (1929), Amsterdam-Z., Wodanstraat 14.
G.W. Versloot (1950), Leiden, Fagelstraat 18.
Dr W. Vervoort (1939), Leiden, Meezenstraat 1.
Dr J.D. Vis (1928), Bergschenhoek, Bergweg 306.
A.H.J. Visschedijk (1952), Utrecht, Pieter Nieuwlandstraat 33 bis.
A. de Visser (1952), Sint Laurens, Noordweg 238.
Dr D. de Visser Smits (1903), Oegstgeest, Hofdijkstraat 36.
Ir J. Vlieger (1933), Leeuwarden, Fred. Ruyschstraat 6.
A.C. van der Vliet (1922), Rotterdam-W., Heemraadsplein 15b.
Dr Ir H. van Vloten (1920), Wageningen, Belmontelaan 5.
Mej. M.C. Vreede (1937), Arnhem, Weg langs 't Hazegrietje 27.
Dr D.M. de Vries (1921), Wageningen, Bowlespark 24.
Dr G.A. de Vries (1944), Baarn, Faas Eliaslaan 21.
H.F. de Vries (1946), Amsterdam-C., Amstel 308 II.
Mej. L.G. de Vries (1950), Utrecht, Willem de Zwijgerstraat 21.
Mej. Dr M.A. de Vries (1945), Leiden Witte Rozenstraat 57.
V. de Vries (1934), Baarn, Torenlaan 16.
M. Vroman (1947), Woerden, Burgem. van Kempensingel 3.
Dr P. van de Vijvere (1952), Brugge (België), Hoogstraat 39.
Dr H.P.J.M. van Waesberghe S.J. (1934), Amsterdam-Z., St Ignatiuscollege, Hobbemakade 51.

- Dr P. Wagenaar Hummelinck (1930), Bilthoven, Beethovenlaan 24.
 Dr J.C.H.J. Wallebroek (1935), Breda, Boschstraat 36.
 Dr J. Wasscher (1934), Aalsmeer, Hortensialaan 25.
 Dr E.C. Wassink (1928), Wageningen, Rijksstraatweg 72.
 H.T.J. Waterbolk (1946), Voorburg, Bruynings Ingenhoeslaan 45.
 Dr Ir S.J. Wellensiek (1928), Wageningen, Diedenweg 10.
 Dr F.W. Went (1922), Pasadena (California, U.S.A.), California Institute of Technology.
 Mej. Dr J.C. Went (1927), Arnhem, Zaanstraat 16 I.
 A. van der Werff (1927), Abcoude, Stationsstraat 1.
 Dr J. Westenberg (1928), Den Helder, Zoölogisch Station, Buitenhaven 27.
 Mej. Dr Joh. Westerdijk (1904), Baarn, Javalaan 20.
 Mevr. A. Westhoff-Morhaus (1951), Utrecht, Rubenslaan 202.
 Dr V. Westhoff (1937), Driebergen, Koninginnelaan 3
 Dr C. Weurman (1949), Cambridge (England), Low Temperature Research Station, Downingstreet.
 Dr H.G. van de Wey (1927), Medan (Indonesia), Deli-Batavia Mij.
 Mevr. Dr N.L. Wibaut-Isebree Moens (1946), Amsterdam-Z., Reinier Vinkeleskade 4 III.
 J.T. Wiebes (1952), Leiden, Van Oldenbarneveldstraat 48.
 Dr L.K. Wiersum (1937), Bogor (Indonesia), Djalan Guntur 12.
 Mej. Dr G. Wilbrink (1904), Lunteren, Dorpsstraat 202.
 Dr J. Wilcke (1946), Bennekom, Hartense weg 22.
 Dr A.G. de Wilde (1948), Leiden, Nieuwe Mare 27.
 Dr L.S. Wildervanck (1946), Groningen, Hooge der A 10.
 Dr F. Wit (1935), Wageningen, Rijksstraatweg 97.
 Dr H.C.D. de Wit (1948), Hilversum, Soestdijkerstraatweg 95.
 K. de Wit (1932), Oosterbeek, Utrechtse weg 140.
 R.J. de Wit (1948), Amsterdam-W., Kijkduinstraat 66 I.
 Mej. U.M.E.A. Witt (1949), 's-Gravenhage, Tapijtweg 4.
 J.R. Wybrans (1938), Medan (Indonesia), Avros Proefstation, Kampong Baru.
 Mr J.H. van Wijk (1946), Bennebroek, Bijweglaan 31.
 Dr R. van der Wijk (1921), Groningen, Mr H. Goeman Borgesiuslaan 20.
 Dr J.S. Zaneveld (1938), Djakarta (Indonesia), Lawangplein 4.
 Mej. Dr J. de Zeeuw (1934), Wageningen, Nassauweg 20.
 W. van Zeist (1948), Groningen, Poststraat 6.
 Dr F.C.J. Zeijlemaker (1938), Amsterdam-C., Prinsengracht 744.
 Dr E.M. van Zinderen Bakker (1929), Bloemfontein (Zuid-Afrika), Universiteitscollege van de Oranje Vrijstaat.
 Dr Ir I.S. Zonneveld (1951), Sleeuwijk, Oud Land van Altenastraat 16.
 L.O. Zwillenberg (1950), Amsterdam-Z., Van Miereveldstraat 9.
 Dr K. Zijlstra (1904), Wageningen, Prof. Ritzema Bosweg 36.

BEGUNSTIGERS

Bedrijfschap voor Sierteeltproducten (1952), 's-Gravenhage.
Centraal Bloembollen Comité Haarlem (1952), Haarlem.
Holland's Bloembollenhuis (1951), Lisse.
N.V. IJsfabriek en Koelhuis IJsvries (1951), IJmuiden.

DONATEURS

N.V. Amsterdamsche Chininefabriek (1952), Amsterdam.
N.V. Rutgerd Veldhuyzen van Zanten Bloembollenbedrijf (1951), Lisse.
Nederlandse Gladiolus Vereniging (1951), Hillegom.
Vereniging de Hyacinth (1951), Haarlem.
Vereniging de Iris (1951), Haarlem.
Vereniging de Narcis (1951), Haarlem.
Vereniging de Tulp (1952), Haarlem.

